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Electrophoretic variations in isoenzymatic pattern of *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae) in relation to host plants and morphs

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ABSTRACT: Several apterous viviparous females of *Lipaphis erysimi* collected from plants of *Brassica juncea*, *Raphanus sativus* and *Rorippa indica indica* in the state of Tripura, North-East region of India, were cloned and investigated genetically by electrophoresis of three enzymes. The aim of this investigation was to find variation, if any, in isoenzymatic pattern of the three enzymes of *L. erysimi* in relation to host plants and morphs. The three host plant-related clones of *L. erysimi* are easily identifiable by observed variation in the pattern of esterase and malic dehydrogenase but not by acid phosphatase. There were no banding pattern differences between the various morphs (nymphs, apterous and alate adults) in the clones using the esterase enzyme. Occurrence of host-specific genetically variable clones of *L. erysimi* confirms an earlier finding which suggested morphological, biological and ecological differences in these aphids. In absence of gene flow between asexual populations of aphids in this tropical part of the world, this result could possibly be explained due to host plant-induced allopatry without geographical barrier.

KEYWORDS: Isoenzymatic pattern, aphids, intra-specific clones

INTRODUCTION

The mustard aphid, *Lipaphis erysimi* (Kaltenbach) continues to be a serious pest of crops of Brassicaceae in tropical and sub-tropical parts of Asia, Africa and America (Agarwala and Bhattacharya, 1999) despite heavy use of pesticides (Bakhetia and Sekhon, 1995). This aphid species is known to infest plants of *Barbrae*, *Brassica*, *Capsella*, *Erysisnum*, *Iberis*, *Lepidium*, *Mathiola*, *Nasturtium*, *Raphanus*, *Rorippa*, *Sinapis*, *Sisybrium*, *Thlaspi*, all belonging to Brassicaceae (Raychaudhuri, 1983; Blackman and Eastop, 1984). Records of chromosomal variation ($2n = 6, 8, 9$, and 10) in the populations of *L. erysimi* from its distribution range suggest that this aphid

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species may be an aggregate of strains or races (Blackman and Eastop, 1984). In an earlier report we showed that host plant-specific clones of *L. erysimi* are distinguishable in morphometry, biology and ecological efficiencies (Agarwala and Das, 1998). In an extention of that work, the second report presents results of electrophoretic study of three enzymes namely, esterase, malic dehydrogenase and acid phosphatase in the aphid clones raised on mustard [*Brassica juncea* (cv 27)], radish (*Raphanus sativus*) and a wild herb, *Rorippa indica indica*. Our working hypothesis was that morphological, biological and ecological distinguishable parthenogenetic clones of *L. erysimi* must be genetically different from each other. We used electrophoresis of enzymes in search of genetic differentiation within *L. erysimi*, since it is an effective and widely used technique (Loxdale *et al.*, 1983; Singh and Cunningham, 1981; Guldemond, 1990). Electrophoretic variation in agricultural pests has been reviewed (Loxdale and Den Hollander, 1989), and a number of enzymes are found to show remarkable isoenzymatic pattern in inter- and intra-specific populations of aphids (Tomiuk and Wohrmann, 1984), Eggers-Schumacher, 1987; Guldemond and Eggers-Schumacher, 1989) and white flies (Wool *et al.*, 1993).

MATERIALS AND METHODS

The study material

Live aphids of *L. erysimi* were collected from assorted plants of mustard, radish and rorippa in rural locations of the Tripura state of north-east India during the winter months of 1997–98. Aphid-infested leaves of the three plants were individually held in 9 cm diameter petri dishes in the laboratory until adult apterous aphids from respective host plants were transferred to pot-grown plants kept in the greenhouse maintained at ambient condition. Sufficient number of plants of the three species were raised from seeds and maintained individually in 10 cm diameter pots. All pots were held in water trays on benches illuminated with sodium vapour lamps. Pots were enclosed with terylene gauze supported by a wooden frame. The colonies of the stock culture were allowed to increase in size without disturbance. Fresh plants of the three species were substituted for any that had deteriorated. This practice allowed an uninterrupted supply of the aphids from the three host species during this study. The study material comprised of parthenogenetic wingless (apterae) females of *L. erysimi*. Aphids of the pot-grown plants of the three species were raised into their standard clones from individual parthenogenetic females. Adequate precautions were taken to protect individual clones from contamination.

Sample preparation

200 mg of fresh adult apterae were obtained from the host-specific clone which meant all individuals of a sample were identical genotypes of one parthenogenetic mother. Aphids were crushed with the help of clay-made mortar and pestle in a mixture of 1 ml of 0.025 M sucrose and 0.10 M TRIS-HCl extraction buffer (pH 6.8). Homogenization was done in freezing condition of ice-bath using a glass homogeniser with teflon

pestle. Homogenised sample was centrifused at 10000 rpm for 20 minutes at 6 °C in a Remi-micro centrifuge. The supernatant of this preparation was stored at −4 °C. 1 drop of glycerol and 0.2% (w/v) bromophenol blue were added to 1 ml of the supernatant as a front-running dye. These were mixed thoroughly. 100 μg of proteins of each sample contained in the mixture was loaded onto a polyacrylamide slab gel pre-soacked in electrode buffer (3.035 g TRIS-HCI, 14.4 g glycine at pH 8.3), using a 7-lane vertical electrophoretor. Gels were generally run for two hours in constant current at 12 mA per gel (6.5 cm).

Preparation of gel

Each time two gels were prepared and used in the electrophoresis. A 8% resolving gel (14.4 cm) was used for the actual run of the sample. Another 4.5% stacking gel was used to filter out any unwanted ingredient which might have got entry during preparation of the sample. 10 ml of 8% *resolving gel* was prepared from 2.7 ml of 30% acrylamide and 0.8% bis-acrylamide in a mixture, 2.5 ml of 1.5 (M) TRIS-HCI buffer (pH 8.8), 4.7 ml double distilled water, 0.006 ml TEMED, and 0.1 ml of 10% ammonium persulphate solution added in sequence. 5 ml of 4.5% *stacking gel* was prepared from 0.83 ml of 30% acrylamide and 0.8% bis-acrylamide in a mixture, 0.63 ml of 0.5 (M) TRIS-HC1 buffer (pH 6.8), 3.45 ml double distilled water, 0.005 ml TEMED, and 0.05 ml 10% ammonium persulphate solution added in sequence.

Preparation of buffers

(a) *Esterase*: 100 ml of 0.1 M Na-phosphate buffer contained 1.21% NaH_2PO_4, $2H_2O$ and 0.28% Na_2HPO_4, anhydrous at pH 6.0.

(b) *Malic dehydrogenase*: 50 ml of 0.1 (M) TRIS-HCL buffer at pH 8.5.

(c) *Acid phosphatase*: 100 ml of 0.5 M acetate buffer contained 9.3% glacial acetic acid and 0.5% NaOH at pH 5.0.

Staining reaction mixture

(a) *Esterase*: In 50 ml of the enzyme buffer, 50 mg of enzyme substrate—naphthyl acetate, 0.6 ml acetone and 0.6 ml distilled water were added to make a reaction mixture. To it was added 50 mg of Fast Garnet GBC salt stain.

(b) *Malic dehydrogenase*: In 7.5 ml of 0.1 (M) TRIS-HC1 buffer following were added in sequence: 62.5 ml double distilled water, 15 mg DL-malic acid, 10 mg NAD, 5 mg NBT, and 10 mg PMS, the staining solution was stored at 37 °C.

(c) *Acid phosphatase*: In 50 ml of the enzyme buffer, 60 mg α-naphthyl acid phosphate and 60 mg of Fast Garnet GBC salt stain was added.

In all the cases staining solutions were filtered to separate undissolved particle of ingredients used in their preparation. The filterate was stored at <5 °C.

Gel staining

Gels were, at first, kept in respective enzyme buffer solutions for 40 min. (Esterase and acid phosphatase samples were kept in freezing condition (<5° C) of a referigerator whereas malic dehydrogenase sample was kept at 37 °C in a room incubator). Thereafter these were transferred to respective reaction mixtures at 37 °C. Reaction time until the bands were resolved were as follows: esterase = 30 minutes; malic dehydrogenase = 4 hrs, and acid phosphatase = 2 hrs.

Mobility of enzymes in gel

The gels were read on an illuminated table. Relative migration was determined by the following method: Rm = distance migrated by specific bands (mm)/distance migrated by marker. After study, gels were fixed in 7% acetic acid at room temperature and sealed in plastic bags.

Data analysis

Data analysis of electrophoresis was carried out at two levels: (i) host plant related inter-clonal comparison of gels, and (ii) intra-clonal comparison of gels obtained from different morphs present in populations comprising of nymphs, apterous (non-winged) and alate (winged) adults. The later study was conducted for one enzyme, esterase, on aphid clones raised on radish and mustard plants. Homogenates of different clones were used in the same run and the relative separation distances of different isoenzyme bands of a given enzyme were obtained in relation to the fastest moving band in that gel. The number of bands and the relative mobility of each band were used as indicators of genetic similarity and difference between the clones of *L. erysimi*. Like most studies of this nature, it was not possible to establish actual genetic bases for the electrophoretic bands observed. It is assumed that when two clones show similar bands, it may or may not be due to a similar genetic differences between them.

RESULTS

Isoenzymatic pattern between the clones raised on three host plant species

L. erysimi is found to be polymorphic for esterase and malic dehydrogenase and monomorphic for acid phosphatase. Electrophoretic mobility of bands of the three enzymes in all the clones from three host plant species of Brassicaceae are presented in Table 1. Esterase is represented by seven isoenzymes, malic dehydrogenase by five isoenzymes and acid phosphatase by a single isoenzymes only.

Esterase. Altogether seven isoenzymes of esterase were found in the clones of *L. erysimi* used in this study. These are designated EST-1 to -7 in order of increasing mobility. Four of the seven loci occurred in all the clones raised on the three host species. These included the weakly stained bands of EST-3, then intensely stained bands of EST-1 and EST-2 and very conspicuous band of EST-7 [Fig. 1(a)]. In

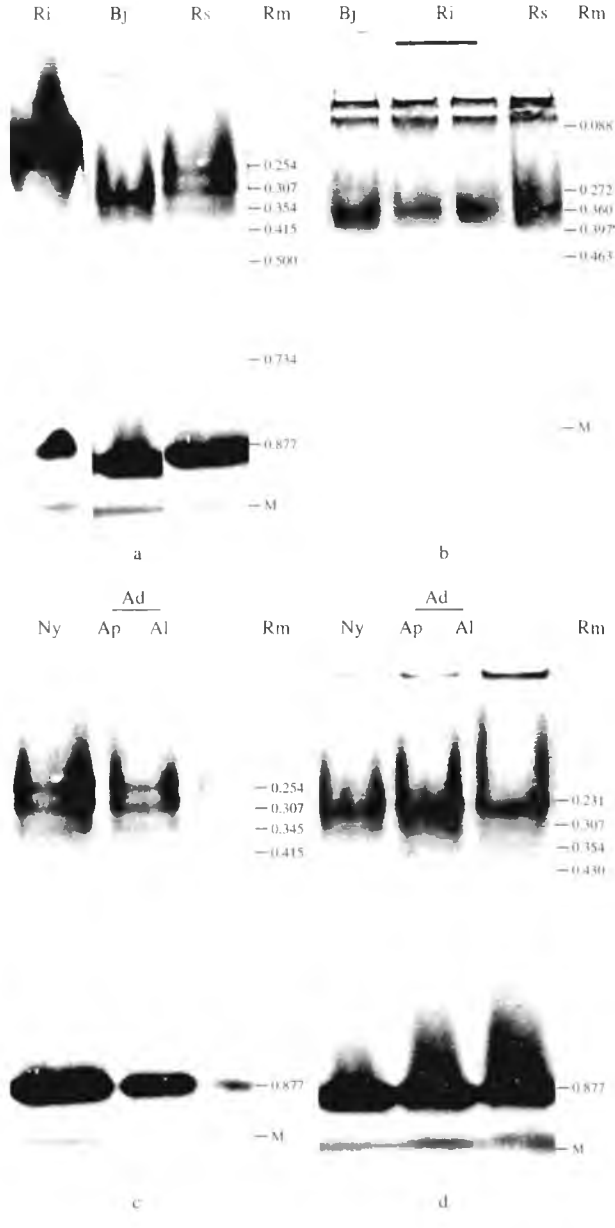

FIGURE 1. Isoenzymatic patterns in *L. erysimi* (a) esterase pattern in relation to host plants-lanes from left: Ri, Bj, Rs = clones from *R. indica indica*, *B. juncea* and *R. sativus*, respectively; (b) malic dehydrogenase pattern in relation to host plants—from left: Bj, Ri, Rs = clones from from *B. juncea*, *R. indica indica* and *R. sativus*, respectively; (c) esterase pattern in morphs of clone from *R. sativus*; (d) esterase pattern in morphs of clone from *B. juncea*. From left: Ny = nymphs, Ap = apterous adults, Al = alate adults.

TABLE 1. Host plant-related electrophoretic variation in relative mobility of bands of three enzymes between clones of *L. erysimi* reared on *B. juncea* (Bj), *R. sativus* (Rs) and *R. indica indica* (Rii)

Locus	Relative mobility of bands in the aphid clones of three host plants		
	Rii	Bj	Rs
(a) Esterase			
EST-1	0.231	0.231	0.254
EST-2	0.312	0.307	0.307
EST-3	0.375	0.354	0.354
EST-4	—	0.430	0.415
EST-5	0.500	—	—
EST-6	0.734	—	—
EST-7	0.890	0.877	0.877
(b) Malic dehydrogenase			
MD-1	0.088	0.088	0.088
MD-2	—	0.294	0.272
MD-3	0.352	0.360	0.360
MD-4	0.404	0.411	0.397
MD-5	0.463	0.455	—
(c) Acid phosphatase			
AP-1	0.307	0.307	0.307

addition, aphid clones raised on rorippa plants exclusively possessed prominent bands at EST-5 and EST-6 whereas the clones raised on radish and mustard plants shared a weakly stained poorly resolved isoenzyme band at EST-4.

Malic dehydrogenase. Five isoenzymes were found in the three clones of this study. These are designated as MD-1 to -5 in order of increasing mobility. All the five isoenzymes were present in the clones raised on mustard plants whereas four were present in each of the clones of radish and rorippa plants [Fig. 1(b)]. Three bands were shared by all the clones from the three host species. These included weakly stained bands of MD-1, and intensely stained bands of MD-3 and MD-4. In addition, aphid clones from mustard and radish plants shared diffuse band at locus MD-2, and weakly stained bands at MD-5 were shared between the clones from rorippa and mustard plants, respectively. Relative mobility of four of these bands, Md-2 to MD-5, showed variation between aphid clones from the three host species. Bands at MD-1 showed uniform mobility in the gel for all the clones of this study.

Acid phosphatase. This enzyme was represented by a sharper band of one locus of uniform mobility (0.37) in the three clones of this study.

TABLE 2. Isoenzymatic pattern of esterase and malic dehydrogenase in different morphs of *L. erysimi* reared on radish and mustard plants

Locus	Relative mobility of bands in the three host plants		
	Nymphs	Apterous adults	Alate adults
(a) on radish plant			
EST-1	0.254	0.254	0.254
EST-2	0.307	0.307	0.307
EST-3	0.354	0.354	0.354
EST-4	0.415	0.415	0.415
EST-5	0.877	0.877	0.877
(b) on mustard plant			
EST-1	0.231	0.231	0.231
EST-2	0.307	0.307	0.307
EST-3	0.354	0.354	0.354
EST-4	0.430	0.430	0.430
EST-5	0.877	0.877	0.877

Isoenzymatic pattern in different morphs

Clone of radish plants. Number of bands resolved between nymphs, apterous and alate adults were the same as recorded for esterase in the adult apterae of the clones raised on the same host in this study (Table 1). In general, slower bands at EST-1 and EST-2 and the fastest bands at EST-5 were intensely stained than the other bands resolved at EST-3 and EST-4. Between the developmental stages, bands of the winged adult aphids were found to be lighter and diffused in comparison to darker and compact bands of nymphs and non-winged adult aphids (Fig. 1C). Otherwise there was no variation in the mobility of different bands between the developmental stages (Table 2).

Clone of mustard plants. Here, also, number of bands resolved between the nymphs, apterous and alate adults were the same as recorded for esterase in the adult apterae of clones raised on the same host in the above study (Table 1). The gels for all the intra-clonal aphids exhibited identical banding pattern (Table 2). In general, slower bands at EST-1 and faster bands at EST-5 were darker and conspicuous in comparison to bands resolved at EST-2, EST-3 and EST-4 which were weakly stained and somewhat diffused [Fig. 1(d)].

DISCUSSION

As this study deals with parthenogenetic clones of a species, therefore, evaluations are based on similarity in band patterns that may or may not have the same genetic basis in clones being compared. Differences in band patterns will suggest genetic differences

between clones; however, a similar band pattern may or may not reflect on similarity in genetic comparison.

The three enzymes used in this study fell into two categories. The first category included acid phosphatase. For this enzyme each of the host plant-related aphid clone in this study had the same one band without any variation in intensity and mobility. It is assumed that this enzyme is equally essential in all the clones. This enzyme is not considered suitable for taxonomic studies. The second category included esterase and malic dehydrogenase. Both the enzymes were represented by multiple loci and showed host plant-related inter-clonal variations in intensity and mobility of bands. Both the enzymes presented diagnostic banding patterns and helped in the separation of aphid clones of *L. erysimi* from the three host plants used in this study. These enzymes are considered useful for taxonomic studies of aphids. Presence of two sharp bands of esterase at loci EST-5 and EST-6 in aphid clones from rorippa plants immediately separates this clone from aphid clones obtained from mustard and radish plants where these bands are absent. Similarly, for malic dehydrogenase, absence of one band each at loci MD-2 and MD-5 from the aphid clones raised on rorippa and radish plants, respectively, separates these clones from each other and also from the aphid clones raised on mustard plants.

Earlier studies have found polymorphic banding pattern for the two forms of esterases in six species of cereal aphids (Loxdale *et al.*, 1983) and white fly, *Bemisia tabaci* (Wool *et al.*, 1993) though the mobility and intensity of resolved bands varied in different species. Singh and Cunningham (1981) also recognised two distinct forms of esterases in six other species of aphids of aphidinae. Each of these aphid species showed characteristic isoenzymes for α-form and β-form considered to represent cholin-esterase and carboxy-esterase, respectively. In the same studies malic dehydrogenase was found to exhibit inter-specific mobility differences in isoenzymes of six species of cereal aphids (Loxdale *et al.*, 1983). In this background, the observed characteristic isoenzymatic pattern for these two enzymes in the three clones of *L. erysimi* vindicate the utility of enzyme electrophoresis in separating animal populations of a species. As regards the isoenzymatic patterns of different morphs of aphids, a few studies that have been made present contrasting results. Castanera *et al.* (1983) failed to find any variation in esterase isoenzyme pattern of apterae and alatae from first to fourth instar nymphs in wheat aphid *Sitobion avenae*. In contrast, Rup and Kalra (1995) showed variation in esterase activity and number of isenozymes in nymphal stages of *L. erysimi*. Results of the present study in having identical isoenzyme patterns in nymphs and adults, both apterae and alate, tend to agree with the findings of Castanera *et al.* (1983). The argument put forward by Rup and Kalra (1995) that metabolic changes associated with differentiation in the first instar and vitellogenesis in adult stages are responsible for variable esterase profile in *L. erysimi* needs to be re-evaluated in the light of the present study. It is not underscored, however, that a firm and reliable genetic relationship at inter-specific and intra-specific levels would require complete data on a large random sample of enzymes including allele frequencies which is often difficult to obtain.

Most of the similar studies in the palearctic and the neoarctic regions on aphids belong to sexually-reproducing species which are characterised by liberal gene flow between populations from different host plants. In contrast, asexual populations of aphids in the hotter parts of Asia and Africa lack gene flow, and the observed host-specialisations in polyphagous and oligophagous species have not been adequately explained. The result of this study tend to suggest that isoenzymatic variations in host-specific populations of an aphid species might be achieved by constant interactions in a specific environment leading to host-induced allopatry without any geographical barrier. Host-induced sympatry has been proposed in sexually reproducing species of *Cryptomyzus* (Guldemond, 1990) which is considered to be the chief means of speciation in these insects (Guldemond and Eggers-Schumacher, 1989). An elaborate study of more enzymes on large samples from wider areas could possibly substantiate the idea of host-induced allopatry in asexual aphids proposed in the above lines.

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Effect of frontal ganglionectomy and administration of precocene II and exogenous juvenile hormone on reproductive behaviour in male *Blattella germanica* Linn. (Blattoidea: Blattellidae)

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ABSTRACT: The neuroendocrine regulation of the sexual behaviour of male cockroach *Blattella germanica* Linn. was investigated with the help of frontal ganglionectomy, chemical inhibition of the corpus allatum and administration of exogenous juvenile hormone. The results revealed that the sexual behaviour was completely blocked following the removal of frontal ganglion. However, the behaviour was only delayed if the activity of the corpus allatum was sought to be inhibited by treatment with low dose of precocene II. Administration of exogenous juvenile hormone brought about resumption of the behaviour, about 24 h after such an administration.

KEYWORDS: *Blattella germanica*, neuroendocrine regulation, sexual behaviour, frontal ganglion, Juvenile hormone analogue

INTRODUCTION

Of various functions of the neuroendocrine system of insects, regulation of sexual behaviour and reproduction in male insects, has received very little attention. It is only in some grasshoppers that this problem has been studied in some depth (Pener and Lazarovici, 1979; Pener *et al.*, 1981; Pener and Shalom, 1987; Shalom and Pener, 1987). Barth and Lester (1973) had pointed out that although cockroaches are a 'most studied' group of insects (because of their easy availability and laboratory culturing), endocrine regulation of their sexual behaviour had remained unexplored. Even though more than a quarter century has elapsed since this observation, not

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much has been added on the subject except for a report of corpus allatum influencing sexual behaviour in male, brown banded cockroach, *Supella supellectilium* (Pathak and Mukerji, 1989). The role of the corpora allata in the male sexual responsiveness has also been demonstrated recently in a lepidopteran *Agrotis ipsilon* (Gadenne *et al.*, 1993). In the present study, neuroendocrine regulation of the sexual behaviour in male German cockroach *Blattella germanica* is investigated.

MATERIAL AND METHODS

Insects

Experimental insects were obtained from the laboratory culture maintained on bread crumbs, milk powder and water *ad libitum*. Male and female adults were separated immediately after the imaginal moult to provide experimental insects of known age.

Observations of reproductive behaviour

Male and female insects of the same age were brought together for experimental pairing on day 5 of the adult life, i.e. after the completion of the prematuration phase lasting for 4 days. Each experimental pairing was carried out with a mature male and a mature female placed together in a glass jar covered with nylon mosquito netting. The paired insects were kept together for 3 h at a time, during which time behaviour of the insects was carefully observed. The following were included in sexual behaviour: (a) attraction for the other sex as shown by the oriented movements, (b) antennation, (c) raising of wings by 80°–85° and stretching of abdomen in males, (d) tapping/licking of the abdomen (feeding on tergal glands?) and thorax of the male by the female while trying to mount the former, (e) mounting, and (f) actual mating. After the pairing time i.e. 3 h at a time, the experimental insects were maintained singly in glass jars. Experimental pairings of the same pair were conducted thrice every week.

Actual time spent on sexual behaviour was converted to per cent of total time spent together in each pairing (i.e. 180 minutes). Means of atleast ten observations of each category of normal/experimental pairings were calculated. These were designated as observational averages. Weekly averages and standard deviations were calculated from this data for three weeks.

Experiments

A. Frontal ganglionectomy

The frontal ganglion was removed in case of male cockroaches on days 0 and 3 of imaginal life. No anaesthesia was used for this operation and the insects were immobilised by wrapping them in nylon mosquito netting and then pinning them on paraffin wax (after Bhandari and Pathak, 1995). The head was then opened with a sharp blade point, to open a window, just between and in front of the two compound eyes. A bit of fat had to be removed to trace the brain. The frontal ganglion located

very superficially, in the intercerebral notch, was sucked out, using a sterilized pipette. A pinch of streptomycin powder was sprinkled over the wound which was then sealed with molten paraffin wax. The operated insects could move immediately, but did not consume any food, although they appeared to nibble at it initially. This was also checked by giving pre-weighed food to these insects, which was again weighed after 30 minutes and after 1 hour. The whole operation from opening of the window on the head, upto closing it after sucking out the frontal ganglion, took less than 5 minutes. Mortality was never more than 20%, though lesser generally, and the surviving insects lived upto 19 days.

Controls consisted of sham operated insects in which window on the head was cut open, and the fat over the brain touched with a sterilized needle, before resealing the wound. The frontal ganglion was not disturbed.

B. Inhibition of the corpora allata

The corpora allata were inhibited by topical application of precocene II (Sigma) in acetone. Precocene II (20 mg), dissolved in 0.75 ml pure acetone giving a final concentration of 26.6 μg/μl, was tightly capped, wax sealed and stored at $-4°$ C. For the purpose of topical application, a 0 day old male insect was immobilised as described above, with ventral side up. A thread in the nylon net was cut over the third and fourth abdominal sterna, observing under a zoom stereoscopic microscope. Using a micropipette, 2 μl of this solution, brought to room temperature, was applied to the exposed sterna. Thus the insect received a little over 53 μg of precocene II. In another group of 0 day old male insects, the dose of precocene II application was doubled by applying 4 μl of this solution. The insects were left immobilised for another 1 minute after the application, before transferring them to their respective containers.

Examination of Paraldehyde fuchsin stained histological preparations of the neuroendocrine system of precocene II treated males (fixed in aqueous Bouin's fluid; one group 4 h post treatment and the other group 5 days post treatment), under light microscope, showed small ($\leq$ 0.2 nl), inactive corpora allata with closely packed nuclei, irrespective of the dose of precocene II administered. The corpora allata of an untreated, freshly ecdysed male were similarly small and with closely packed nuclei. However, the glands of a 5 day old, untreated male were always much larger ($\geq$ 0.3 nl) with plenty of cytoplasm.

Sham operations consisted of application of pure acetone to male insects on the day of eclosion and at the age of 5 days.

C. Allatectomy

Allatectomy was also performed in 12 freshly moulted male insects, which were immobilised and a window was cut in the neck region. The corpora allata were removed using a sharp, hooked needle. The neck was resealed using molten paraffin wax, after sprinkling a pinch of streptomycin powder over the wound. Eight of the allatectomised insects died. Sham operations were performed wherein the same procedure was repeated except that the corpora allata were not removed.

D. Administration of exogenous juvenile hormone

Juvenile hormone III (Sigma) was dissolved in pure olive oil (P. sassoefigli, Italy) to give a concentration of 25 μg per μl, tightly capped, wax sealed and stored at -4° C. The solution was discarded if not used within 10 days. Administration consisted of injecting 2 μl of the solution after bringing it to room temperature, in the 3rd/4th abdominal segments of an immobilised insect, using a microsyringe (Hamilton).

Sham administration consisted of injection of 2 μl of pure olive oil.

JH treatments were given to the insects in the following manner: (i) 0 day old normal males, (ii) 10 day old male insects subjected to frontal ganglionectomy at the age of 0 day, (iii) 4 day old male insects treated earlier with precocene II at the age of 0 day, (iv) 7 day old male insects treated earlier with precocene II at the age of 0 day, and (v) 9 day old male insects treated earlier with precocene II at the age of 0 day. Experimental insects of categories (iii) and (iv) included those given single or double dose of precocene II while insects of category (v) were provided only single dose of precocene II.

E. Experimental design to study sexual behaviour

The following types of males were paired with females to study the sexual behaviour:

(i) Normal
(ii) Frontal ganglionectomised (FG^{-}) and controls
(iii) FG^{-} + JH III and controls
(iv) Insects treated with precocene II (both doses, given on day 0) and controls and
(v) precocene II + JH III and controls (if available).

RESULTS

Effect of frontal ganglionectomy

The effect of frontal ganglionectomy on the sexual behaviour of male *Blattella germanica* is presented in Table 1. No behaviour was shown by the operated males, irrespective of the day of operation i.e. day 0 or 3. Unoperated and sham operated individuals showed normal sexual behaviour. Administration of JH III on day 10 in frontal ganglionectomised males caused the resumption of sexual behaviour in about 24 hours of the administration. In such cases, the per cent time spent on sexual behaviour was less than 50% of that shown by unoperated and sham operated males. This however, gradually increased and was noted to be much higher towards the end of second and the beginning of the third observational week.

Effect of treatment with precocene II on the corpora allata

Treatment with precocene II on day 0 of adult life of *Blattella germanica* always resulted in small corpora allata, the size being similar to that seen in newly ecdysed males. The glands looked shrunken and the insects showed no sexual

TABLE 1. Observational averages of per cent time (minutes) spent on sexual behaviour by male *Blattella germanica* after the removal of frontal ganglion on day 0 and day 3. Counting of observational weeks was started from day 5 onwards after imaginal moult

S. No.	Treatments	Observational averages of per cent time spent (minutes) on sexual behaviour ±SD								
		First week			Second week			Third week		
		1	2	3	4	5	6	7	8	9
1	Frontal ganglionectomy on day 0; FG^-, d 0.	0	0	0	0	0	0	0	0	0
2	Frontal ganglionectomy on day 3; FG^-, d 3.	0	0	0	0	0	0	0	0	0
3	Sham Operated* on day 0	0	3 (0.14)**	10.42 (0.39)	58.6 (1.3)	83.1 (0.68)	12 (0.14)	14.22 (0.47)	46.21 (1.2)	4.32 (0.56)
4	Frontal ganglionectomy on day 0; followed by administration of JH III on day 10; FG^-, d 0 plus JH^+, d 10	0	0	0	25.11 (1.41)	66 (1.56)	55 (2.02)	26.21 (2.2)	37.23 (1.96)	2.05 (0.55)

* Frontal ganglion exposed, but not disturbed, wound resealed. ** Figures in parenthesis indicae S.D.

behaviour (Table 2). It was noted that the males treated with 2 μl of the chemical (i.e. single dose, containing 53.3 μg of precocene II), showed no sexual behaviour up to the end of the second week of observations but resumed the same after a week. On the other hand, males treated with 4 μl of the solution (i.e. double dose, containing 106.6 μg of precocene II) did not show any sexual behaviour even upto the end of third observational week, of the period for which observations were made. It appears that the effect of precocene II in inhibiting the activity of the corpora allata and the sexual behaviour was dose dependent and lasted for about two weeks if the dose administered
was about 50 μg, but was more prolonged, if the dose was doubled. Sexual behaviour was also not seen in allatectomised males. But administration of exogenous juvenile hormone to all these categories of males i.e. precocene II treated (both single or double dose) or allatectomised, brought about the resumption of sexual behaviour in about 24 hours.

DISCUSSION

Frontal ganglionectomy resulted in two effects in male *Blattella germanica*; it inhibited the sexual behaviour and adversely affected the feeding. Roussel (1964) had reported cessation of feeding following frontal ganglionectomy in *Gryllus maculatus*, but there are no reports of inhibition of sexual behaviour as a result of the removal of this ganglion.

Another observation that merits attention is the presence of a direct nerve connection between the recurrent nerve coming from the frontal ganglion and the corpora cardiaca (CC) in many insects e.g. *Bombyx mori* (Bounhiol *et al.*, 1953), *Hypera postica* (Tombes, 1972), *Manduca sexta* (Bell *et al.*, 1974) and *Supella supellectilium* (Pathak and Mukerji, 1984). Clarke and Anstee (1971a,b) had found that in *Locusta* the removal of the frontal ganglion not only silenced the endocrine system and suppressed the release of the neurosecretory material (NSM), but also resulted in abnormal and completely inactive corpora allata (CA). The authors suggested that the frontal ganglion controlled the release of the NSM from the CC. Pathak and Mukerji (1989) had reported the influence of the CA on the sexual aggressiveness and reproductive behaviour of the male *Supella*.

Two possibilities emerge from these observations. Firstly, it appears that the NSM was released from the CC only when a neural/hormonal signal was received from frontal ganglion. The released NSM then activated the CA which synthesised and released the juvenile hormone (JH) which in turn triggered the sexual behaviour, provided the stimulus for the same, in the form of sex pheromones of a mature female, was available in the vicinity. Following the frontal ganglionectomy, the series of actions failed to occur and trigger the sexual behaviour even when the stimulus was available in the vicinity. The second possibility is that starvation, resulting from the cessation of feeding following frontal ganglionectomy, adversely affected the internal nutritional milieu which is known to affect the functioning of the CA (Engelmann,

TABLE 2. Observational values of per cent time spent (minutes) on sexual behaviour by allatectomised. precocene II treated and JH III treated males of *Blattella germanica*. Counting of observational weeks was started from day 5 after imaginal moult

S.No.	Treatments	Observational averages of per cent time spent (minutes) on sexual behaviour ±SD								
		First week			Second week			Third week		
		1	2	3	4	5	6	7	8	9
1	Control	0	4.17 (0.29)	10.56 (1.2)	62.5 (2.51)	99.17 (2.16)	9.72 (1.61)	11.33 (2.22)	52.11 (2.2)	3.89 (1.07)
2	$Preco^{+}$ d 0	0	0	0	0	0	0	41.85 (1)	72.22 (1.94)	22.32 (2.15)
3	$Preco^{++}$ d 0	0	0	0	0	0	0	0	0	0
4	$Acet^{+}$ d 0	0	2.52 (0.52)	15 (1)	50.56 (1.64)	75.11 (2.07)	20.56 (2.6)	8.89 (1.88)	29.5 (1.89)	7.67 (1.99)
5	JH^{+} d 0	11.67 (1.8)	38.52 (1.91)	58.33 (2.22)	11.11 (1.91)	13.89 (2.53)	12.22 (2.42)	16.67 (2.63)	47 (2.67)	16.67 (3.19)
6	$Preco^{+}$ d 0; JH^{+} d 4	0	3.06 (1.04)	9.29 (1.69)	39.81 (3.03)	61.11 (3.17)	11.11 (2)	*	0	0
7	$Preco^{+}$ d 0; Olive oil d 4	0	0	0	0	0	0	29.01 (2)	31.55 (2.57)	20 (2.17)
8	$Preco^{+}$ d 0; JH^{+} d 7	0	0	3.06 (0.89)	35.56 (3.3)	58.33 (2.62)	8.33 (1.74)	0	0	0
9	$Prec^{+}$ d 0 JH^{+} d 9	0	0	0	8.7 (2.69)	66.11 (3.7)	18.33 (1.74)	0	0	0
10	CA^{-} d 0	0	0	0	0	0	0	0	0	0
11	CA^{-} d 0; JH^{+} d 7	0	0	5 (1.22)	42.16 (2.52)	48.88 (2.07)	10.6 (2.16)	0	0	0

* Not observed; $Preco^{+}$ Treated with precocene II in acetone (Single dose); $Preco^{++}$ Treated with precocene II in acetone (Double dose); $Acet^{+}$ Treated with acetone only; JH^{+} Treated with JH III in Olive oil; CA^{-} Allatectomised; Figures in parentheses indicate S.D.

1970). As a result of non-activation of the CA, the synthesis and release of the JH was either reduced or was totally absent and the sexual behaviour was not triggered.

Although, it is still not clear how the frontal ganglion is related to the activation of the CA, it does appear from the present observations that the release of JH, triggered the sexual behaviour, as administration of exogenous JH did result in resumption of this behaviour.

In order to confirm the involvement of CA and JH in triggering the sexual behaviour, the CA were either removed or chemically cauterised using precocene II. It was found that each of these actions resulted in absence of sexual behaviour, which was however, resumed within 24 h of administration of exogenous JH analogue.

But the fact that a low dose of precocene II resulted in automatic resumption of sexual behaviour in third week of observations, was surprising. Brooks *et al.* (1979) and Pratt *et al.* (1980) had earlier reported that a reactive intermediate, formed *in situ*, selectively destroyed the CA. How did the CA escape destruction in this case to be able to synthesise and release JH for automatic resumption of sexual behaviour? It appears that the action of precocene II was dose dependent. Zamorano *et al.* (1981) had also found that a dose of less than 100 μg given to the fourth instar nymphs was not effective in causing sterility in emerging female *Blattella*. Belles and Messeguer (1981) had also noted in the same species that even a dose of 200 μg applied to a five day old female caused only a temporary sterility; most experimental insects becoming fertile once again at third/fourth oothecal stage. Pratt and Pener (1983) have also discussed the relative sensitivity of *Locusta* female adults to precocene II and it's dose dependent action. Feyereisen *et al.* (1981) had also reported the dose dependent effect of topical application of precocene II on the inhibition and destruction of CA in females of *Diploptera punctata*. It appears that a dose of about 50 μg of precocene II caused only a temporary inhibition of sexual behaviour in male *Blattella*, while a dose of over 100 μg resulted in a much more prolonged loss of such behaviour. Although all these results discussed here, refer to female insects and the endocrine action need not be the same in the two sexes, it appears that the dependency on dose of precocene II is not restricted to females only.

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Triacylglycerol lipase activity during adult development of armyworm, *Mythimna separata* (Walker)

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ABSTRACT: The changes in triacylglycerol lipase activity during adult development of *Mythimna separata* have been studied. The partial characterization of enzyme revealed maximum activity at pH 8.0, temperature 37° C, 30 min incubation time, 1 per cent enzyme concentration and 5 per cent substrate concentration. The Km for triacylglycerol lipase from adult was found to be 0.266×10^{-2} mM. In case of fed moth, gradual increase in enzyme activity from 1 to 2-day, sharp increase from 2 to 4-day, gradual decrease from 4 to 7-day and sharp decrease from 7 to 9-day male adults was observed. In case of female gradual increase in enzyme activity from 1 to 2-day, sharp increase from 2 to 5-day and slow decrease from 5 to 9-day female adults was observed. The enzyme activity in male during early adult development was more as compared to female, while during later development it was more in female as compared to male. In case of starved moth gradual increase in the enzyme activity from 1 to 3-day and decrease from 3 to 6-day male and female adults was observed. The enzyme activity in male during early adult development was more as compared to female. The physiological significance of triacylglycerol lipase during adult development of *Mythimna separata* is discussed.

KEYWORDS: Triacylglycerol lipase, adult development, armyworm, *Mythimna separata* (Walker)

INTRODUCTION

The armyworm, *Mythimna separata* (Lepidoptera: Noctuidae) is an economically important polyphagus pest of the graminaceous crops like maize, sorghum, sugarcane, fingermillet, rice and wild grasses in India. The larval stage being nocturnal, it feeds voraciously at night on green foliage of host plants leaving behind only the bare midribs. In case of wheat, rice and millets the larvae cut the base of earhead of panicles

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leading to great losses in yield. The adult developmental period of *M. separata* is of 9 days in fed moth and 6 days in starved moth.

During larval feeding period, deposition of all mass necessary for the final adult development takes place. Lipids are of vital importance to many insects as substrates for embryogenesis, metamorphosis and flight (Gilbert, 1967). Fat is the chief nutrient form in which energy is stored. It is usually present in greatest amount in the mature larva before metamorphosis (Wigglesworth, 1972). The major lipid component of the insect fat body is long chain fatty acid triglyceride (Gilby, 1965). For utilization of lipids especially triglycerides, for energy production, the component of fatty acids must be hydrolised and triacylglycerol lipase (3 : 1 : 1 : 3) is the enzyme which is responsible for such hydrolysis (Nandanan *et al.*, 1973).

The flight of an insect involves very rapid oxidation of respiratory fuels by the flight muscle (Bailey, 1975). Lipids, carbohydrates and, in some insects, certain amino acids are used as respiratory fuels to supply the energy for flight (Sacktor, 1965, 1970). Flight muscles contain enough fuel to initiate flight but the endogenous reserves are too small to sustain prolonged flight for which respiratory fuels must be supplied to the muscles from exogenous sources. The flight muscles derive their exogenous supply of respiratory fuels from the haemolymph, which itself contains an important reserve of organic materials. However, the major energy storage site in the insect is the fat body which is often considered the equivalent of the mammalian liver and adipose tissue combined and which may contain large quantities of glycogen, triglycerides and proteins (Kilby, 1965).

A number of workers have investigated indetail various aspect of *M. separata*, mainly efficacy of different insecticides for control of *M. separata* (Deol *et al.*, 1981), nuclear polyhedrosis virus in *M. separata* (Neelgund and Mathad, 1972), use of pheromone traps to control *M. separata* (Liu *et al.*, 1985), bionomics of *M. separata* in laboratory and field conditions (Singh and Rai, 1977) etc. but the biochemical and physiological aspects of this animal are yet to be studied. The triacylglycerol lipase is very important enzyme in insects which hydrolise triglycerides and release energy and supply structural components during development. However, the information on the triacylglycerol lipase during adult development of *M. separata* is rather scanty. There exists a lacuna in the field of triacylglycerol lipase activity during adult development of *M. separata* which is mainly concerned with release of energy for sustained flight in both the sexes and material necessary for oogenesis in female moth. Therefore the present study attempts to provide information on triacylglycerol lipase activity during adult development of *M. separata*.

MATERIALS AND METHODS

The culture of *M. separata* was maintained in the laboratory on the natural food of cut pieces of maize leaves. The egg masses were put in the watch glass. The eggs were regularly observed and black egg masses were kept on small pieces of young maize leaves for hatching. Every alternate day the food of larvae was changed. The pupae were collected and kept in glass jar lined with black paper at its inner side

for the emergence of moths. Newly emerged male and female moths obtained from pure culture were confined in the big jar in ratio 2 : 1, containing folded paper for oviposition. Cotton wad dipped in 10 per cent honey solution was provided as a food for the moths. The food was changed twice a day. To study the adult development without food the newly emerged moths were confined in another jar and were starved giving no food or water. The eggs laid on the paper were collected and kept in watch glass. Thus the culture of *M. separata* was maintained at room temperature.

All the solvents were of reagent grade and were obtained from E. Merck and Co. Rahway N.J., USA and BDH, England. Unless otherwise indicated solvents were redistilled in the laboratory under anhydrous condition before use. Diphenyl carbazid was purchased from E. Merck, Dermstat, Germany. Diphenyl carbazone was of Veb Jenapharm Laborchemie, Apolda, Germany. Triolein and Stearic acid were obtained from Sigma Chemicals Company, USA. Tris-HCI, Tris-Maleate and all other chemicals were of the highest purity commercially available.

For the enzymatic preparation, the moths were repeatedly washed with distilled water and dried on filter paper under ceiling fan for homogenization after removal of their antennae, wings, and legs etc. The fed and starved male and female moths weighed and homogenized separately in cold double distilled water using a ground glass mortar and pestle. The homogenates were diluted with cold double distilled water so as to get 1 per cent concentration. Such homogenates were used for the biochemical assay of the enzyme activity. The triacylglycerol lipase was assayed by the method of Hayase and Tappel (1970). The assay system contained 0.25 ml 5 per cent of substrate dispersed in gum acacia, 1.0 ml of 0.1 M tris-maleate buffer pH 8.0 and 0.25 ml of 1 per cent enzyme solution in a total volume of 1.5 ml. The incubations were carried out in a shaker with a continuous shaking for 30 minutes in glass stoppered vessels at 37 °C. At the end of the incubation the liberated fatty acids were measured colorimetrically according to Itaya (1977).

RESULTS

Adult developmental period of *M. separata* is of 9 days in fed moth and 6 days in the starved moth. The moths are very energetic having strong power of flying. The number of eggs laid per female has been recorded to be 500 to 550. The mating is observed in 2, 3 and 4-day adult developmental period.

The partial characterization of triacylglycerol lipase during adult developmental revealed the maximum activity at pH 8.0, temperature 37 °C, 30 minutes incubation time, 1 per cent enzyme concentration and 5 per cent substrate concentration. The Km for triacylglycerol lipase from the adult of *M. separata* was found to be 0.266×10^{-2} mM.

The changes in triacylglycerol lipase activity during adult development of fed and starved moths of *M. separata* are shown in Fig. 1. In case of fed moth, gradual increase in enzyme activity from 1 to 2-day, sharp increase from 2 to 4-day, gradual decrease from 4 to 7-day and sharp decrease from 7 to 9-day male adults was observed. Maximum activity was observed in 4-day male adults. In case of female gradual

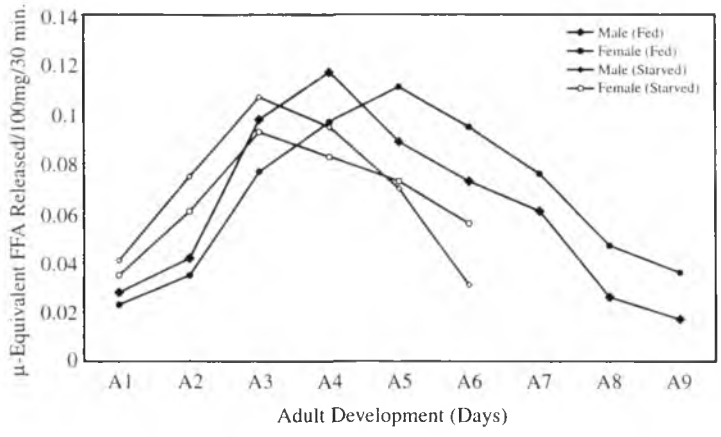

FIGURE 1. Triacylglycerol lipase activity during adult development of *M. separata.*

increase in enzyme activity from 1 to 2-day, sharp increase from 2 to 5-day and slow decrease from 5 to 9-day female adults was observed. Maximum activity was observed in 5-day female adults. The enzyme activity in male during early adult development was more as compared to female, while in later period it was more in female as compared to male.

In case of starved moth gradual increase in the enzyme activity was observed from 1 to 3-day and decrease from 3 to 6-day male and female adults. Maximum activity is observed in 3-day male and female adults. The enzyme activity in male during early adult development was more as compared to female.

DISCUSSION

The hydrolysis of triglycerides clearly showed the presence of triacylglycerol lipase in the adult homogenate of *M. separata*. Similar observations were reported in the adult homogenates of *Chilo partellus* by Pol and Sakate (2001).

Lipids are very suitable energy sources in insects since an isocaloric quantity of triglyceride occupies much less storage space than the equivalent amount of glycogen (Bailey, 1975). The initiation and maintenance of insect flight require a highly efficient oxidative metabolism which is fueled by carbohydrates and lipids. Insect may use different energy sources according to the stage of flight. For example, although the oxidation of fatty acids provides the energy for sustained flight in locusts, carbohydrate is used as a readily mobilized substrate to initiate flight (Chippendale, 1978). Jutsum and Goldsworthy (1976) have shown that adult males of *Locusta migratoria* rely upon energy derived from the oxidation of carbohydrates during the first 30 min of flight, when haemolymph trehalose is oxidized at a rate of 120 μg/min. After 30 min of flight, energy was shown to be provided mainly by fatty acid oxidation Stevenson (1972) studied the haemolymph lipid pattern of moths flown for 30 min in southern

armyworm, *Prodenia eridania*. He reported the triglyceride concentration reduced by about one half and the monoglyceride and free fatty acid concentration somewhat increased. He also reported that *Prodenia* flight muscle contains very little lipid; most of this may be structural lipid, and not metabolically available. The fat body is the chief lipid depot in the insect, and its neutral glyceride is almost all triglyceride. The fat body contains enzymes able to hydrolyse tri-, di- and monoglycerides which indicates that triglyceride can be completely hydrolysed in the fat body and the resulting free fatty acids were carried to the flight muscles to provide energy for flight.

Fat body can synthesize lipids from non-lipid precursors and the fat body triglycerides whatever their origin can be mobilized and transported to the flight muscles to act as a respiratory fuel (Bailey, 1975). Lipase plays a key role in the mobilization of energy (Krysan and Guss, 1973). In migratory moths, it appears that there is no sexual dimorphism in lipid content since both sexes migrate and presumably use equal amounts of lipid during flight (Gilbert, 1967). Adults of *M. separata* survived for more than 6 days without food, suggesting that they could migrate for more than 2 days. The ovaries were not mature at emergence but began to develop when the female fed on honey solution. Female assumed the calling position and mated when they had mature eggs (on and after day 3). The day on which the pairing occurs was delayed when the female had been starved on days 1 and 2, but there was no difference between the total fecundity of fed and starved females. The results suggest that the incipient stimulus to migration would be flight for feeding (Hirai, 1984). Pol and Sakate (2001) studied adult lipase activity of *Chilo partellus*. They noted the adult developmental period of *Chilo partellus* is of 4 days and during adult development the gradual increase in enzyme activity from 1 to 2-day, maximum activity in 2-day and gradual decrease from 2 to 4-day adults.

In the present work in case of fed moth gradual increase in triacylglycerol lipase activity was observed from 1 to 2-day male and female adults of *M. separata*. This indicates that carbohydrate catabolism predominates during early adult development as a source of energy to initiate flight. Sharp increase in enzyme activity from 2 to 4-day male adult and 2 to 5-day female adults suggests the lipid especially triacylglycerol catabolism which provides energy to sustained flight for migration, search of food and mating. As moths of *M. separata* are migratory, they could migrate more than two days and covers considerable distance. Maximum activity was observed in 4-day male adults and 5-day female adults. It indicates maximum utilization of lipids for release of energy for sustained flight in both the sexes and extensive oogenesis in female. The extensive oogenesis requiring essential structural components and energy is derived from breakdown of lipid and mainly from triglycerides.

The enzyme activity in male during early adult development is more as compared to female indicates active role of male in search of virgin female and mating. Gradual decrease in enzyme activity from 4 to 7-day male adults indicates less energy requirement after mating. Sharp decrease in enzyme activity from 7 to 9-day male adults may be due to depletion of lipid and inactive physiological state of male.

Slow decrease in enzyme activity from 5 to 9-day female adults suggests release of energy for oogenesis and oviposition. The enzyme activity in female during later adult development is more as compared to male indicates active role of female in oogenesis and oviposition.

In case of starved moth gradual increase in enzyme activity from 1 to 3-day male and female adults suggests the lipid especially triglyceride catabolism which provides energy to sustained flight for migration, search of food and mating in both the sexes. Maximum activity was observed in 3-day male and female adults. It indicates maximum utilization of lipids for release of energy for sustained flight in both the sexes and extensive oogenesis in female. The enzyme activity in male during early adult development is more as compared to female indicates the active role of the male in search of virgin female and mating. Gradual decrease in enzyme activity from 3 to 6-day male and female adults indicates less energy requirement after mating in male and for oviposition in female.

During adult development the hydrolysis of lipids mainly triglycerides takes place which provides energy for sustained flight for migration, search of food and mating in both the sexes and material for oogenesis in female. The main source of energy during adult development is lipid and triacylglycerol lipase activity is instrumental in release of energy. Our findings agree with the findings of Gilbert (1967), Stevenson (1972), Krysan and Guss (1973), Bailey (1975), Jutsum and Goldsworthy (1976), Chippendale (1978), Hirai (1984) and Pol and Sakate (2001).

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Predatory efficiency of *Cybister confusus* (Coleoptera: Dytiscidae) on developmental stages of a major carp *Catla catla* Ham.

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ABSTRACT: The paper deals with the result of predatory efficiency of three different length and body weight groups of both sexes of *Cybister confusus* Sharp on spawn and fry stages of *Catla catla* Ham, a major carp of freshwater system in laboratory. The highest predation capacities (N/hr) on maximum spawn (80) densities were recorded as 10.40 ± 1.12, 10.10 ± 1.36, 12.40 ± 0.78 for male and 13.40 ± 1.09, 14.10 ± 1.12, 12.20 ± 1.36 for female beetle on the spawn of *Catla*. The predation potentialities on maximum fry densities (80) for male were 5.00 ± 0.82, 5.40 ± 0.40, 5.80 ± 0.78 and for female beetle as 4.20 ± 0.78, 4.80 ± 0.41, 6.10 ± 0.99. The rate of predation was highest on maximum prey density (80) while it was reduced as the size of prey increased. It was also found that the rate of predatory efficiency was more in female than the male beetle.

KEYWORDS: Predatory efficiency, *Cybister confusus* Sharp, spawn, fry, *Catla catla* Ham.

INTRODUCTION

Considerable observation has been made on the role and interaction of predatory aquatic insects with the fish spawn, fry and fingerlings by the various fishery biologists as well as entomologists in India (Pakrashi, 1953; Alikunhi *et al.*, 1955; Julka, 1965; Roy, 1990; Sinha, 1992). All these works have been done under field conditions except the work of Sinha (1992) and literature pertaining to predatory efficiency of aquatic insects on fish spawn, fry, tadpoles, plankton and macro-invertebrates in the laboratory are rather scanty. Practically no information is available on the prey destroying capacity and stage specific mortality caused to major carps by these predatory aquatic insects.

Cybister confusus Sharp is the largest carnivorous aquatic beetle and occupies top position in the food chain of the freshwater ecosystems. The developmental stages

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(spawn and fry) of major carps (*Catla catla*, *Labeo rohita*, *Cirrhina mrigala*) usually encountered a strong biotic resistance from this voracious predator who directly causing injuries to them and also consume their natural food. Therefore, in order to know the predatory efficiency of *Cybister confusus* Sharp on various developmental stages of *Catla catla* Ham. in the laboratory, the present study has been undertaken.

MATERIALS AND METHODS

The experiment was conducted in the laboratory during rainy season of the year 1996 when spawn and fry of various economically important major carps are available. *Cybister confusus* Sharp was collected from the local fish ponds, brought live in the laboratory and maintained in glass aquaria. The aquaria were provided with aquatic macrovegetations making the medium as nearly natural as possible. All glass aquaria were filled with fish pond water. After one week maintenance of this beetle in the laboratory, the experiment was conducted by selecting six adults (three males and three females) of different length and body weight groups. The beetles were separately placed in glass aquaria ($20 \times 20 \times 15$ cm). The beetles kept starved for 24 hrs and then exposed to their prey. They were fed with spawn and fry of *Catla* at five different prey densities (5, 10, 20, 40 and 80). All predation trials were carried out at room temperature. The number of prey killed by predator species was noted for one hour. This experiment was repeated five times using the same process of predation trials. The predation trials were repeated after the gap of 24 hrs and trials were observed for only one hour. The prey-predator relationship was studied following the pattern of Ellis and Borden (1970) with some modifications.

RESULTS AND DISCUSSION

The predatory efficiencies of *Cybister confusus* Sharp on different prey densities (5, 10, 20, 40 and 80) of both spawn and fry of *Catla catla* Ham. in the laboratory were recorded and presented in Table 1 for spawn and Table 2 for fry respectively. The mean values of maximum spawn consumption (N/hr) by three specimens of different length and body weight groups of both male and female *C. confusus* Sharp on higher prey densities (80) were recorded as 10.40 ± 1.12, 12.10 ± 1.36, 12.40 ± 0.78 for male while 13.40 ± 1.09, 14.10 ± 1.12, 12.20 ± 1.36 for female beetle respectively. In case of fry, the mean values of consumption (N/hr) by both sexes of the same predator on higher prey density of fry (80) were 5.00 ± 0.82, 5.40 ± 0.41, 5.80 ± 0.78 for male and 4.20 ± 0.78, 4.80 ± 0.41, 6.10 ± 0.99 for female respectively.

It was observed that in almost all predation trials, the predator insect species of different length and body weight groups of both sexes had different prey consumption capacity. The rate of predation was gradually increasing as the density of prey increased. The results of mean values of consumption (N/hr) by three different length and body weight groups of both sexes of predator *C. confusus* Sharp on minimum and maximum (5 and 80) prey density of *Catla* spawn recorded were 1.20 ± 0.75, 1.80 ± 0.61, 2.40 ± 0.49 and 10.40 ± 1.12, 12.10 ± 1.36, 12.40 ± 0.78 for male and

TABLE 1. Predatory efficiency (N/hr) of different length, body weight and sex group of *Cybister confusus* Sharp on spawn densities of *Catla catla* Ham.

Utilization of spawn (N/hr.) $N' = 5$

Prey Density	*C. confusus* Sharp (Male) I (30 mm) (3.0 gm)	II (38 mm) (4.0 gm)	III (40 mm) (4.5 gm)	*C. confusus* Sharp (Female) I (35 mm) (3.5 gm)	II (45 mm) (4.2 gm)	III (48 mm) (5.0 gm)
5	1.20 ± 0.75	1.80 ± 0.61	2.40 ± 0.9	3.10 ± 0.59	3.40 ± 0.49	4.00 ± 0.82
10	3.60 ± 0.46	3.70 ± 0.58	5.20 ± 0.78	3.50 ± 0.47	3.70 ± 0.78	6.40 ± 1.02
20	3.80 ± 0.41	4.80 ± 0.41	7.80 ± 1.17	7.10 ± 0.93	8.00 ± 1.27	8.50 ± 1.03
40	8.20 ± 0.89	9.20 ± 1.09	9.00 ± 0.81	9.40 ± 1.12	10.00 ± 1.45	11.80 ± 1.08
80	10.40 ± 1.12	12.10 ± 1.36	12.40 ± 0.78	13.40 ± 1.09	14.10 ± 1.12	12.20 ± 1.36

ANOVA

Source of variation	D.F.	SS	MSS	F	SS	MSS	F
Subgroup	14	1004.78	71.77		1071.966	76.569	
A	2	46.32	23.16	21.43^a	18.04	9.02	6.89^a
B	4	928 ± 0.04	232.01	214.73^b	1001.56	250.39	191.45^a
A × B	8	30.413	3.80	3.51^b	52.35	6.544	5.00^a
Error	60	64.8265	1.0804			78.4705	1.30784
Total	74	1049.606				1150.437	

[a] $P < 0.01$ A: Predator (*Cybister confusus* Sharp; Male & Female)

[b] $P < 0.05$ B: Prey (*Catla catla* Ham. spawn) Density

N' = Five predation trials

TABLE 2. Predatory efficiency (N/hr.) of different length, body weight and sex group of *Cybister confusus* Sharp on Fry densities of *Catla catla* Ham.

Utilization of spawn (N/hr.) $N' = 5$

Prey Density	*C. confusus* Fry (Male) I (30 mm) (3.0 gm)	II (38 mm) (4.0 gm)	III (40 mm) (4.5 gm)	*C. confusus* Sharp (Female) I (35 mm) (3.5 gm)	II (45 mm) (4.2 gm)	III (48 mm) (5.0 gm)
5	1.00 ± 0.38	1.30 ± 0.61	2.20 ± 0.79	1.20 ± 0.75	1.50 ± 0.49	1.80 ± 0.61
10	2.40 ± 0.49	2.60 ± 0.84	2.80 ± 0.64	2.80 ± 0.84	2.20 ± 0.79	3.00 ± 0.69
20	3.00 ± 0.69	3.40 ± 0.49	3.50 ± 0.47	3.90 ± 0.59	3.50 ± 0.47	5.00 ± 0.82
40	4.20 ± 0.79	4.30 ± 0.89	4.60 ± 0.81	4.80 ± 0.41	5.00 ± 0.78	5.20 ± 0.78
80	5.00 ± 0.82	5.40 ± 0.40	5.80 ± 0.78	4.20 ± 0.78	4.80 ± 0.41	6.10 ± 0.99

ANOVA

Source of variation	D.F.	SS	MSS	F	SS	MSS	F
Subgroup	14	113.68	8.12		173.78	12.4133	
A	2	4.38	2.19	3.77^a	9.80	4.90	7.99^b
B	4	104.58	26.14	45.11^b	160.15	40.90	65.24
A × B	8	4.723	0.59	1.06^b	3.826	0.478	0.7795
Error	60	34.77	0.579		36.817	0.613616	
Total	74	158.451			210.6036		

[a] $P < 0.01$ A: Predator (*Cybister confusus* Sharp; Male & Female)

[b] $P < 0.05$ B: Prey (*Catla catla* Ham. Fry) Density

N' = Five predation trials

3.10 ± 0.59, 3.40 ± 0.49, 4.00 ± 0.82 and 13.40 ± 1.09, 14.10 ± 1.12, 12.20 ± 1.36 for female respectively. The rate of predation efficiency was more in females than in males.

It was observed that both sexes of beetle caused maximum damage to spawn and to a lesser extent inflicted injuries to fry stages. No damage is caused to fingelings stages of carp by this predatory insect. Peckrasky (1982) reported that a predator prefers certain size of the prey and are known to consume them in higher proportion, as they are relatively abundant in nature. In the present study the rate of predation by *Cybister confusus* Sharp increased, as the density of prey also increased and maximum predatory efficiency was found on (80) prey density of the spawn of *Catla*.

In the ANOVA Table values 'A' indicates the consumption capacity of three specimens of different length and body weight groups of both sexes of *Cybister confusus* Sharp. The value were found significantly different at 1% level of significance in almost all predation trials. The values 'B' indicates the effect of different prey density (5, 10, 20, 40 and 80) of spawn and fry on *C. confusus* Sharp. This value was again found significantly different at 1% level of significance. The values of A × B indicates the interaction between predatory beetle (A) and prey spawn and fry (B). The present computation of data on predatory efficiency and prey annihilation indicates that the prey density was the prime factor in increasing the predation rate. Thus, the prey consumption capacity is directly dependent upon their density in the habitat (Sinha, 1992).

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Seasonal incidence and biology of the mango hopper *Amritodus atkinsoni* Lethier (Homoptera: Cicadellidae)

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ABSTRACT: Studies on seasonal incidence, biology, morphometrics and ovipositional behaviour of mango hopper, *Amritodus atkinsoni* Lethier carried out in Tirupati region of Andhra Pradesh during 1998–99 are reported. Mango hoppers were found colonized during both vegetative and reproductive phases of the crop. Peak incidence was noticed during full bloom stage of the crop after which the insects later migrated to cracks and crevices of the tree trunk. The biology includes an incubation period of about four to eight days, four nymphal stages lasting for 10 to 14 days and adult longevity for three to four days. The eggs were deposited singly on the midribs of the tender leaves, flower buds and inflorescence. The distal portion on the lower surface of the leaf was the most preferred site for oviposition. The eggs were deeply thrusted into the tissue singly in a row and remain exposed in the slit made during oviposition.

KEYWORDS: seasonal incidence, biology, morphometrics, mango hopper, *Amritodus atkinsoni* Leth.

INTRODUCTION

Mango hoppers are widely distributed in all mango growing regions of the country causing serious threat to mango production. The loss due to mango hoppers may go upto 60 per cent. Among many species of hoppers infesting mango, *Amritodus atkinsoni* Leth. is a predominant one which caused frequent outbreaks in Chittoor district, a major mango growing region of the state. Though many workers (Dalvi and Dumbre, 1994; Hiremath and Hiremath, 1994; Patel *et al*, 1994) have provided data on the morphometrics and seasonal occurrence of the pest, information pertaining to its development under local environment is lacking. Hence detailed studies on the biology and morphometrics of *Amritodus atkinsoni* L. were carried out.

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MATERIALS AND METHODS

To study the seasonal incidence of the mango hopper, *A. atkinsoni* Leth. observations at fortnightly intervals during August 1998 to July 1999 were made on five trees, selected in the middle of the orchard. From each tree, four branches representing each direction were selected for recording the data. Again within each branch, five twigs were selected and tagged for making the hopper count. The samples were collected in a polythene bag, haaving a cotton swab dipped in chloroform. For collecting the insects, the selected twig or inflorescence was inserted into the bag and tapped gently so that the hoppers including both nymphs and adults fall in it, later the hopper count was recorded.

Collection and maintenance of hopper culture

To document the biology of *A. atkinsoni*, adult hoppers were collected and released into twig cages that were prepared by making holes on either side of the plastic jar (20 × 9 cm), one end, of which was closed with a layer of muslin cloth in order to provide free circulation of air and allow the moisture inside the jar to get evaporated. Subsequently, the development of hoppers from egg stage onwards was documented in specially made rearing chambers under laboratory conditions at a mean temperature of 28 ± 2 °C. The rearing chambers were prepared with petriplates which consists of a thin layer of cotton of 0.5 cm thickness, spread inside the petriplate over which a filter paper was placed. The cotton layer inside was always kept moist continuously. These rearing chambers were placed in an insect rearing cage maintained at room temperature of 28 × 2 °C.

The leaves and panicles were exposed to gravid hoppers for egg laying for two days and kept on filter paper in the rearing chambers. The observations were made twice a day to note the incubation period. After hatching, the neonate nymphs were transferred onto fresh inflorescence in the rearing cage. Data on time taken for the development of nymph and adult transformations were recorded. The differences in growth stages were qualified by recording morphometrics in terms of length and width of the egg, body length and width of nymphs across the thorax and body width across the compound eyes.

RESULTS AND DISCUSSION

Seasonal incidence

Incidence of mango hoppers was observed throughout the year. The peak incidence and activity of the hoppers coincided with vegetative and reproductive phases of the crop. However, they remained stable in cracks and crevices of the tree trunk during the absence of flowering panicles. The level of hopper incidence gradually decreased from 21.6 to 15.6 from August to September. It again started increasing to higher levels during October (38.4 hoppers) which reached to its peaks level (92.4 hoppers) by Februvary. From second fortnight of February, when flowering started declining, the hoppers slowly migrated to cracks and crevices of the trunk. The

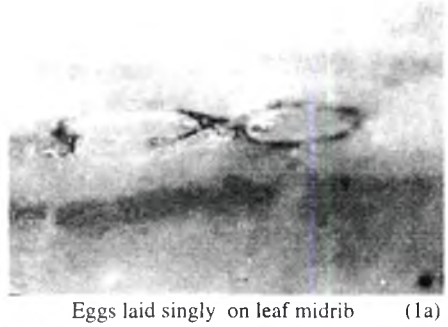
TABLE 1. Seasonal incidence of Mango hoppers *Amritodus atkinsoni* during August 98 to July 99

Sl. No.	Fortnight	Months	Levels of Hoppers* (No./twig/pancle/ 10 cm^2 of trunk)
1.	I	August	21.6
2.	II	August	15.6
3.	I	September	14.4
4.	II	September	15.6
5.	I	October	38.4
6.	II	October	54.0
7.	I	November	57.6
8.	II	November	57.6
9.	I	December	80.4
10.	II	December	78.0
11.	I	January	79.20
12.	II	January	91.20
13.	I	February	92.40
14.	II	February	128.95
15.	I	March	135.75
16.	II	March	137.00
17.	I	April	135.00
18.	II	April	113.31
19.	I	May	88.21
20.	II	May	68.35
21.	I	June	48.00
22.	II	June	20.50
23.	I	July	0.0
24.	II	July	0.0

* Mean of 5/10 spots.

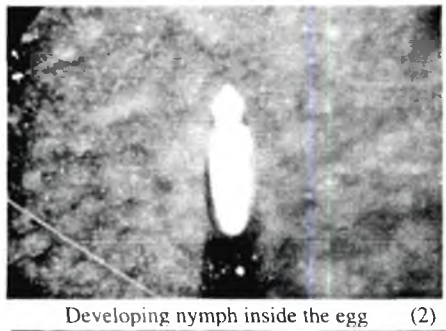
hopper population was 128.95 per cm^{-2} which reached to a maximum of 137.00 by March (Table 1). Again from April, the population started declining in number which almost disappeared recording their stray movement on the plant. From September onwards, hoppers started their activity coinciding with new vegetative flush. The same trend continued upto June with the increase in percentage of flowering (Fig. 1). The three major hoppers infesting mango crop in Chittoor district are *A. atkinsoni* Leth, *Ideoscopus niveosparsus* L. and *I. clypealis* L, though the former was predominant. The present observations of peak hopper incidence during vegetative and reproductive stages of crop coincide with those of Dalvi and Dumbre (1994); Hiremath and Hiremath (1994), and Patel *et al* (1994). However, hoppers became less active and took shelter in the cracks and crevices of the trunk in the absence of both vegetative and flowering phases of the plant.

A positive relation was found between hoppers and maximum temperature (r = 0.1696) while these were found negatively related to minimum temperature (r = 0.463); rainfall (r = 0.391) and relative humidity (r = 0.4077) (Table 2). These



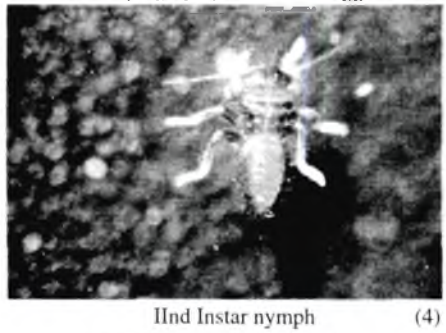
Eggs laid singly on leaf midrib (1a)

Individual eggs (1b)

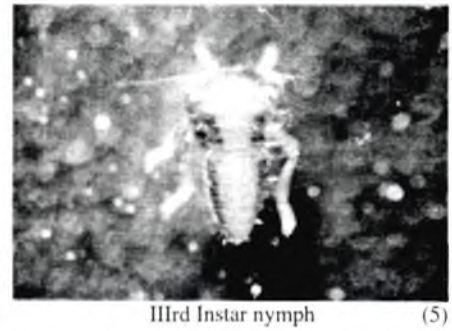


Developing nymph inside the egg (2)

Instar nymph emerging from egg (3)



IInd Instar nymph (4)



IIIrd Instar nymph (5)

IVth Instar nymph (6)

Adult of *Amritodus atkinsoni* (7)

PLATE I. Figures 1–7 showing Biology of Mango hopper, *Amritodus atkinsoni* L.

TABLE 2. Relationship of infestation of mango hoppers with weather parameters

Weather parameter	Correlation coefficient(r)	r table value at 5%
Maximum Temperature	−0.16956	0.285
Minimum Temperature	−0.463	
Rainfall	−0.3910	
Relative humidity	−0.4077	

studies are not comparable with those of Sood *et al* (1971) and Sheikh *et al* (1993) who reported that minimum temperature of 20 °C affect the activity of the pest, since under the present ecosystem, the temperature rarely go down to the extent of 20 °C.

Biology

Egg

The eggs are laid mainly on new vegetative flush or inflorescence stalks. On the twigs, the lower surface of leaf was preferred more than the upper surface. The mean number of eggs laid per floral stalk and twig were 115.2 and 130.6 respectively. The eggs are smooth, dull white, translucent and oval in shape. Later on, they turned yellowish. Eggs are somewhat pointed at one end and blunt at the other end. After hatching, an empty egg shell was found with an opening on the blunt end. Individual egg on an averege measured about 1.038 mm in length and 0.273 mm in width. On the leaf surface/vein, the mean number of eggs per leaf on distal, middle and proximal portions were 6.1547, 1.9906 and 0.8264 respectively. After oviposition, the leaf tissues surrounding the eggs became dark brown in colour and leaf tips were found drooping. The incubation period varied from 4 to 8 days with an averege of 5.9 ± 0.41 days (Table 3) (Figs 1a & b).

I instar

The newly emerged nymph was pinkish brown in colour which later changed to pale brown, bulged red compound eyes and long black bristles on the abdomen could be seen prominently. Neonate nymph has an averege body length of 1.060 mm and width of 0.431 mm in prothorax, 0.386 mm on mesothorax and 0.342 mm in metathorax. The width across the eyes measured about 0.477 mm. The size of the head was found to be bigger than rest of the body. The average duration of the nymph remained as 2.2 ± 0.13 days (Figs 2 & 3).

II instar

This instar was initially pale brown in colour but later changed to dark brown with big body size. The abdominal segments appeared clearly with reduced bristles. The developmental period of the nymph lasted for three to four days with an averege of 3.5 ± 0.16 days (Table 3). The body length was 1.492 and width was 0.540 mm on prothorax, 0.485 mm on mesothorax and 0.379 on metathorax respectively (Fig. 4).

TABLE 3. Growth parameters of different life stages of mango hopper *A. atkinsoni* L.

Sl. No.	Stage	Measurement (mm)*			Duration in days
		Minimum	Maximum	Average	
1.	Egg				5.9 ± 0.41 (4–8)
	a. Length	0.009	1.101	1.039	
	b. Width	0.218	0.333	0.273	
2.	I Instar				2.2 ± 0.13 (2–3)
	a. Body length	0.767	1.380	1.060	
	b. Body width	0.368	0.537	0.431	
	ii. Mesothorax	0.322	0.445	0.386	
	iii. Metathorax	0.276	0.383	0.342	
	c. Head width	0.383	0.721	0.477	
3.	II Instar				3.5 ± 0.16 (3–4)
	a. Body length	1.334	2.116	1.492	
	b. Body width				
	i. Prothorax	0.457	0.567	0.540	
	ii. Mesothorax	0.429	0.521	0.485	
	iii. Metathorax	0.307	0.475	0.379	
	c. Head width				
	(Across the eyes)	0.521	0.675	0.572	
4.	III Instar				2.6 ± 0.15 (2–3)
	a. Body length	2.530	2.914	27.60	
	b. Body width	2.530	2.914	2.760	
	i. Prothorax	0.767	0.905	0.837	
	ii. Mesothorax	0.675	0.874	0.759	
	iii. Metathorax	0.613	0.813	0.679	
	c. Head width				
	(Across the eyes)	0.920	1.135	1.028	
5.	IV Instar				3.6 ± 0.15 (3–4)
	a. Body length	2.914	3.604	3.047	
	b. Body width				
	i. Prothorax	0.797	1.304	1.181	
	ii. Mesothorax	0.736	1.196	1.086	
	iii. Metathorax	0.736	1.119	0.986	
	c. Head width				
	(Across the eyes)	1.564	1.656	1.619	
6.	**Adults**				3.5 ± 0.16 (3–4)
	A. Males				
	a. Length	4.200	5.000	4.630	
	b. Head width (Across the eyes)	1.733	1.840	1.777	
	B. Female				
	a. Length	4.500	5.100	4.910	
	b. Head Width (Across the eyes)	1.856	2.055	1.951	

*Average of 10 samples

III instar

After eclosion of the old skin, the nymph was dark brown in colour with clearly differentiated abdominal segments and well developed wing pads on thoracic segments. The developmental period lasted on an average of 2.6 ± 0.15 days. The nymph measured about 2.760 mm in length and 0.387 mm width on prothorax, 0.759 mm in mesothorax and 0.679 mm on metathorax. The width of head across the eyes measured an average of 1.028 mm (Fig. 5).

IV instar

Immediately after molting, the body of the nymph was pale brown in colour which later became dark brown. This instar resembled the adults in all aspects except for wing development. The insect at this stage, exhibited large wing pads with active diagonal movement like an adult The duration for development lasted on an average of 3.5 ± 0.16 days (Table 2). The body has an average length of 3.047 mm and width of prothorax 1.181 mm, on mesothorax 1.086 mm and 0.986 mm on metathorax. The width of the head across the compound eyes measured about 1.619 mm on an average (Fig. 6).

Adult

The fully matured adults were light brown in colour, which were found moving actively from one twig to other. Adults have two sports on the scutellum. The females differed from males by having sickle shaped ovipositor. The longevity on an average was 3.5 ± 0.16 days. The females measured about 4.91 mm in length where as males about 4.63 mm on average. The width of the head across the compound eyes was 1.951 mm in females and 1.777 mm in males (Fig. 7).

The documentation made by earlier workers like Hiremath and Thontadarya (1999) coincide with the present investigations recording 4 to 8 days of incubation period. Similarly, the morphology of the nymphal stages exhibiting the long black coloured bristles on abdomen and differentiation of abdominal segments in advanced nymphal stages coincide with the observations of Patel *et al* (1975) from South Gujarat. The total nymphal period varying from 10 to 14 days are in conformity with previous observations of Hayes (1970); Butani (1979) and Hiremath and Thontadarya (1999). The body length of male and female remained the same as observed by Butani (1974) ranging from 4.2 to 4.8 mm and 4.7 to 5.1 mm respectively and total life circle from17 to 26 days (Patel *et al*, 1975). But these are not comparable to the observations made by Atwal (1963) and David and Kumaraswami (1988). The contradictions in the inference made by these workers when compared to the present investigation may be due to the variation in growth conditions or influence of environmental factors of the region.

The newly emerged adults of mango hoppers were found to be dark brown in colour which more quickly from one twig to other. Adults posess two spots on scutellum region (Srivatsava, 1997). The body length of male and female confirm with previous observation (Butani, 1974) who has reported them as ranging from 4.2 to 4.8 mm and

4.7 to 5.1 mm respectively. The man width across the compound eyes in males and females was measured as 1.777 and 1.951 mm which differed slightly from that of Viraktamath (1997). Similarly adult longevity varied from 3 to 4 days which differing from that of Hiremath and Thontadarya (1999) who have reported the adult longevity as 3 to 8 days. However, according to Patel *et al* (1994) the adults survived for 3 to 4 days on natural food under laboratory conditions. The total life cycle of A. atkinsoni varied from 17 to 26 days including 4–8 days of incubation period and 10–14 days of nymphal period. Similer observations reported by Patel *et al* (1975) and Hiremath and Thontadarya (1999) under laboratory conditions, but differed from those of David and Kumaraswami (1988). The contradictions in the inferences made by these researchers when compared to the present results, underline the influence of environmental factors of a region and their impact on biology of the mango hoppers.

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Efficiency of digestion of oligosaccharides in the gut and salivary gland of some seed feeding lygaeids (Heteroptera: Lygaeidae)

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ABSTRACT: A qualitative profile of the exoglycosidases in the digestive tract of three lygaeids species namely *Spilostethus hospes* (Fab), *Spilostethus pandurus* (Scopoli) and *Caenocoris nerii* (Germar) is provided based on the hydrolysis of the α and β-linked oligosaccharides. The various region of the alimentary tract where activities of α and β-galactosidases and α and β-glucosidases were observed is provided, *C. nerii* did not show β-glucosidase activity while β-galactosidase was absent in both the species of *Spilostethus*. The efficiency of digestion of the oligosaccharides by the three lygaeid species is compared in terms of the number of glucose molecules liberated over a period of time. The importance of study of the β-glucosidase activity in the development of new pest management strategies is discussed.

KEYWORDS: exoglycosidases, α and β-linked oligosaccharides, α and β-galactosidases, *Spilostethus hospes*, *Spilostethus pandurus*, *Caenocoris nerii*

INTRODUCTION

Glycosidases are hydrolytic enzymes catalyzing glycosidic linkages, which link a carbohydrate to a peptide, to a lipid, or to another carbohydrate. Exoglycosidases act on only the terminal non-reducing monosaccharides of poly or oligo-saccharides. They are relatively specific for the sugar to be released as well as the configuration (α or β) of the bond (Flowers and Sharon, 1979). Most of the naturally occurring plant glycosidases are β-linked O-glycosil compounds as such β-glycosidases appears important in the metabolism of plant glycosides (Ahmad *et al*, 1986; Yu, 1989). Members of the Asclepidaceous family are known for their cardiac glycosides content which are especially concentrated in the seeds. The lygaeid bugs *Spilostethus pandurus* (Scopoli), *Spilostethus hospes* (Fab) and *Caenocoris nerii* (Germar) show specific preference for feeding on the seeds of the *Calotropis gigantea* (L.) (Sanjayan

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and Ananthakrishnan, 1987). Cardenolids from such host plants are sequestered by lygaeids throughout their life cycle (Duffey and Scudder, 1974; Moore and Scudder, 1985) and the insect suffers no apparent ill effects (Chaplin and Chaplin, 1981). Although insects β-glycosidases are capable of hydrolyzing glycosides to release carbohydrates, the allelochemical aglycones that are released during this process become toxic to the insects. Therefore β-glycosidases may work to their disadvantage when they ingest plant glycosidases. Herbivorous insects become enzymatically adapted to phytochemicals present in host plant (Ahmad, 1986). Little work has been carried out on the digestive glycosidases and most of them relate to flies or mosquitoes which can feed on sugar or nectar or on leaf feeding insects (Burgess *et al*, 1990). The present work describes and characterizes the exoglycosidases of the salivary gland and gut of some seed-feeding lygaeid bugs.

MATERIALS AND METHODS

Rearing of insects

Spilostethus pandurus, *S. hospes* and *C. nerii* were collected from *C. gigantea* and mass reared in plastic jars measuring 20 cm × 10 cm and the mouth was covered with the help of a muslin cloth. Seeds of *C. gigantea* and a cotton wad dipped in water used as a source of moisture was provided.

Enzyme preparation

Ten numbers of adult insects were separated from the mass culture. They were then etherized and dissected under ice cold phosphate buffer saline of pH 7.0. The alimentary canal was freed of adhering trachea and the different regions including the salivary gland were severed, weighed and homogenized separately. The midgut was divided into three, namely midgut-I, midgut-II, and midgut-III and IV combined (Sanjayan, 1985). The homogenate was centrifuged at 26 000 g for 20 min using a Remi refrigerated centrifuge. The volume of the resulting supernatant was adjusted to read 5 mg tissue/ml was used for the enzyme analysis.

Enzyme mixture

The reaction mixture contained 0.4 ml of 0.1 M phosphate buffer at pH 7.0 (0.05 M) 0.2 ml of enzyme extract at a concentration of 5.0 mg tissue/ml, 0.2 ml of 1% substrate and a drop of toluene as an antiseptic. For each carbohydrate tested, a control sample was prepared. The test and control samples were incubated at 37 °C for one hour.

Detection of enzyme activity

Enzyme activity was determined under optimal conditions using 3, 5-dinitrosalicyclic (DNS) acid reagent which determine the free aldehydic groups of glucose formed after the digestion of respective substrates. After incubation, the reaction was terminated by the addition of 1.6 ml of DNS reagent. The mixture was heated for 5 min at 100 °C in a water bath and then cooled in an ice bath and diluted with 1.6 ml of diluted water.

The reduced DNS acid was measured using Hitachi Model U3210 spectrophotometer at an absorbency of 550 nm.

RESULTS

Qualitative profile of digestive enzymes of lygaeids

The α and β-linked oligosaccharides, Cellobiose, Melibiose, Raffinose and Lactose were provided as substrates and their hydrolysis tested at various region of the alimentary tract of three lygaeid species namely *S. pandurus*, *S. hospes* and *C. nerii* (Table 1). Cellobiose is hydrolyzed by β-glycosidase but *C. nerii* could not hydrolyze it in its alimentary canal. On the contrary, *S. hospes* hydrolyzed the oligosaccharide in its alimentary tract and salivary gland, whereas *S. pandurus* showed weak capacity to hydrolyze it through the secretion of the salivary gland.

Alpha-galactosidase brings about the digestion of raffinose and melibiose. While raffinose was hydrolyzed in the midgut of *S. hospes*, the insect did not hydrolyze melibiose. On the other hand, *S. pandurus* efficiently utilized raffinose and melibiose, the former being hydrolyzed throughout the midgut, while the later being digested by the salivary secretions and partly by the midgut-III and IV. *C. nerii* showed a strong capacity to hydrolyze melibiose through the secretion of the salivary gland and the midgut-III and IV while raffinose was weakly hydrolyzed in the rear midgut region.

No evidence of the presence of β-galactosidase that hydrolyzes lactose was in the alimentary canal of the lygaeids *S. pandurus* and *S. hospes*. However, the salivary secretions of *C. nerii* showed a distinct activity of this enzyme.

Quantitative determination of enzyme efficiency and compartmentalization of the digestive process

Figure 1 provides a comparative picture of the efficiency of digestion of the α- and β-linked oligosaccharides by the different lygaeid species and also depicts the various regions of the alimentary tract where the hydrolysis takes place. Of significance is that though the three lygaeid species feed on the seeds of *C. gigantea*, there appears to be a physiological resources partition in respect of the utilization of the various nutrients. Each species to be efficient in utilizing one or the other type of oligosaccharide. *S. hospes* utilized cellobiose and to a lesser extent raffinose; *C. nerii* melibiose; *S. pandurus* raffinose while lactose was hydrolyzed only by *C. nerii*. The digestion of these oligosaccharides was and large in the midgut, with the midgut-III and IV showing greater hydrolytic activity than the comparatively larger midgut-I and II regions. The salivary gland secretion of *S. hospes* digested cellobiose although greater hydrolysis took place in the midgut-III and IV. Lactose was totally hydrolyzed by the salivary secretion of *C. nerii* and absolutely no digestion of lactose was observed in the midgut. In all the lygaeid species studied, the hindgut showed no enzymatic activity except for *S. hospes* which had β-glycosidases digesting the cellobiose sugars.

The efficiency of digestion of the oligosaccharides by the three lygaeid species could be compared in terms of the number of glucose molecules liberated in the

TABLE 1. Hydrolysis of some oligosacchrides in different region of the alimentary tract of the three lygaeid species

Region	Cellobiose β-glucosidases			Melibiose α-glucosidases			Raffinose α-galactosidase			Lactose β-galactosidase		
	P	H	N	P	H	N	P	H	N	P	H	N
Salivary gland	1.4 ± 0.22	8.02 ± 0.36	0	5.54 ± 0.26	0	35.0 ± 3.40	0	0	0	0	0	17.66 ± 2.17
Midgut-I	0	2.04 ± 0.28	0	0	0	3.6 ± 2.4	4.6 ± 1.35	2.16 ± 0.26	0	0	0	0
Midgut-II	0	3.8 ± 0.28	0	0	0	4.2 ± 2.56	4.35 ± 1.35	3.28 ± 0.29	0	0	0	0
Midgut-III & IV	0	14.2 ± 0.4	0	5.34 ± 0.21	0	36.4 ± 4.12	11.0 ± 1.41	3.86 ± 0.28	0	0	0	0
Hindgut	0	10.0 ± 0.56	0	0	0	0	0	0	0.66 ± 0.26	0	0	0

P – *S. pandurus*, H – *S. hospes*, N – *C. nerii*. Values represent mean of 5 replicates ± SD Values are in μg glucose liberated/hr.

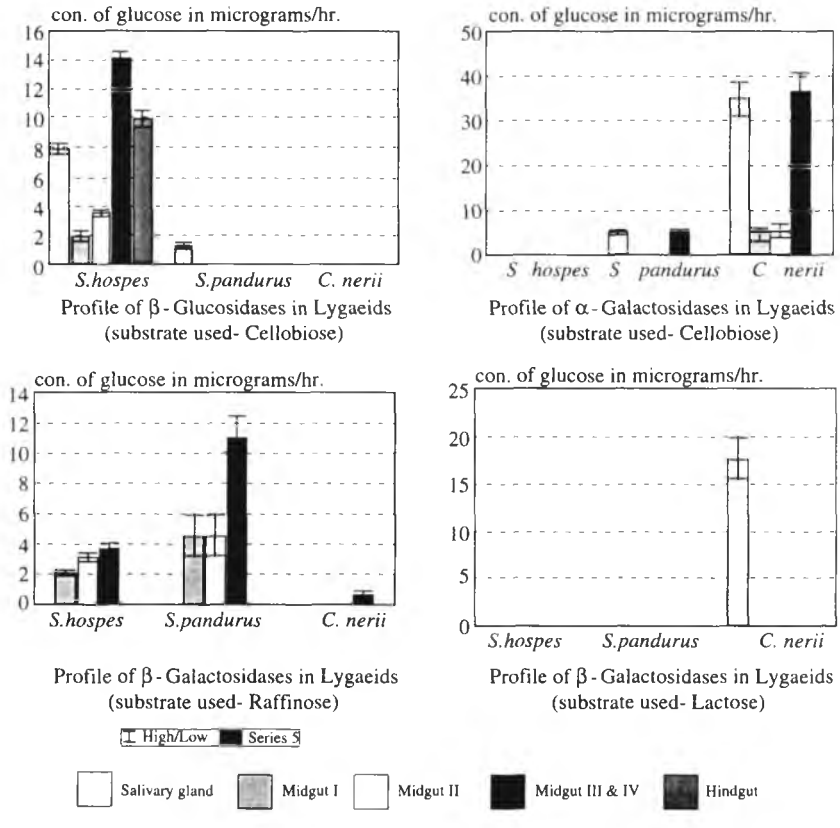

FIGURE 1. Comparative efficiency of digestion of oligosaccharides at different regions of the alimentary canal of three lygaeid species.

TABLE 2. Quantitative efficiency of glucose liberation upon digestion of three lygaeid species

Species	Substrates				
	Cellobiose	Melibiose	Raffinose	Lactose	Total
S. hospes	37.8 ± 1.08	0	9.3 ± 0.59	0	47.1
S. pandurus	1.4 ± 0.22	10.88 ± 0.36	20.2 ± 0.97	0	32.48
C. nerii	0	79.2 ± 11.01	0.66 ± 0.26	17.66 ± 2.17	97.52

Values represents mean of 5 replicates ± SD; Values are in μg of glucose liberated/hr.

alimentary canal over a period of time (Table 2). 38.06 ± 1.08 μg of glucose was liberated by *S. hospes* through digestion of cellobiose which was significantly greater (1.4 μg) than that released by *S. pandurus*. Similarly, *C. nerii* significantly released

more glucose upon digestion of melibiose than *S. pandurus*. The amount of glucose liberated by *S. hospes* from digestion of raffinose *C. nerii* released the maximum glucose from digestion of melibiose but could also hydrolyze lactose from which it released 17.6 ± 2.17 μg of glucose which the other lygaeid species failed to utilize.

DISCUSSION

Most lygaeid bugs are specifically seed-feeders and this ability require them to have a good complement of digestive enzymes so as to bring about easy hydrolysis of the complex nutrients that characterize seeds. In this study we have made *in vitro* analysis of three species of lygaeids for their capacity to hydrolyze the α and β-linked oligosaccharides cellobiose, melibiose, raffinose and lactose. The exoglycosidases namely, the α and β-galactosidas and β-glucosidase bring about the hydrolysis of these oligosaccharides. Our studies indicate that these enzymes are present in the three lygaeid bugs, but β-glucosidase was not present in *C. nerii* and β-galactosidase that hydrolyze melibiose was not present in *S. hospes*. The exoglycosidases were mainly distributed in the midgut tissues, although some activity was also observed in salivary glands. *S. hospes* showed a moderate hydrolysis of cellobiose in the hindgut. Although mid gut of insects is the principal site where digestive enzymes are secreted (Wigglesworth, 1972), enzymes are also present in the hindgut (Partho *et al*, 1992). Also in all the three lygaeid species studied, greater activities of these enzymes were observed in the midgut-III and IV regions compared to the midgut-I and II. In terms of the efficiency of liberating glucose molecules upon hydrolysis of the oligosaccharides, *C. nerii* was more efficient than the *S. hospes* and *S. pandurus*. However each species showed individually in their capacity to hydrolyze one or the other type of oligosaccharide efficiently.

The glycosidases represent the water soluble forms of the aglycone allelochemical and are usually non-toxic. While α-glucosidase releases the useful carbohydrates from sugar with α-linkages, the β-glucosidases hydrolyze sugar residues from β-glucosidic allelochemicals, the major storage forms in plants. This is clearly done at the risk of releasing the toxic aglycone. Interestingly, β-galactosidase is present only in the salivary glands of *C. nerii* and absent in the other two species of *Spilostethus*. β-glucosidase is totally absent in *C. nerii* and present in small quantities in the salivary glands of *S. pandurus*. This indicates that the present of β-glucosides only in the salivary glands should probably work with an adaptive advantage in utilizing the carbohydrates even if they are glucosidically attached to an allelochemical since in this case the action of the enzyme would cleave the nutrient molecule from the allelochemical which could then be subsequently sucked by the bug. In this process of the action of the enzyme, the allelochemical, which was hitherto soluble while in combination with the glycone molecule, becomes insoluble and is left in the seed, not to be sucked by the bug thereby preventing toxicity.

Beta-glucosidases appear to be active both in the salivary gland as well as the midgut of *S. hospes*. This action of the enzyme is definitely done at the risk of the allelochemical being released into the body of the insect. However, no toxicity was

observed probably because of some detoxifying enzymes mechanisms present in the insect. The MFO are among the important detoxifying enzymes available in insects (Ahmad *et al*, 1986; Applebaum, 1985) which *S. hospes* may be making use of. Alternatively lygaeid bugs particularly those belonging to the subfamily lyaeinae to which *S. hospes* belongs, have the capacity of sequestering the allelochemical and utilizing it for protection against predators (Duffey and Scudder, 1974). Contrary to the β-glycosidase profile of the gut, the α-glycosidases are found in most insects (Wigglesworth, 1972). β-glycosidases are less common and reported in only a few species such as *Periplaneta americana*, *Aphis fabae*, *Callosobruchus maculatus* (Gatehouse *et al*, 1985). *Chrysolinina* species (Randox *et al*, 1990) and *Maduca sexta* (Ahmad and Hopkins, 1992). These enzymes occur in several isoenzymic forms and may be able to split several different sugars (Chiponlds and Chararas, 1985). However, considerable specificity is indicated for plant β-glucosidases (Hosel and Conn, 1982). The insect alimentary microflora also provides a major source of the enzyme. Low level of β-glucosidases and high level of α-glucosidase were recorded for the potato aphids (Mullin, 1986). In the present study, *S. hospes* alone showed more of β-glucosidase activity whereas both *S. pandurus* and *C. nerii* had more of α-glycosidase activity. The study of β-glucosidase in phytophagous insects is important not only in understanding the biochemistry of the insect, but also in developing new pest management strategies. Plants produce various allelochemicals as defensive weapons among which the glycosides seem to play a very important role in host plant resistance to insects. The toxic action of the glucosidase could be due to their corresponding aglycones liberated by the action of β-glucosidase. Therefore high β-glucosidase activity is detrimental to phytophagous insects feeding on plants with toxic allelochemicals. If we know the substrate specificity of β-glucosidase and the consequences of the hydrolysis in a phytophagous insect, we can utilize this knowledge to develop plant varieties with more plant defensive systems.

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Seasonal occurrence of the mulberry leaf-roller, *Diaphania pulverulentalis* (Hampson) and its parasitoids

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ABSTRACT: The leaf-roller, *Diaphania (Margaronia) pulverulentalis* (Hampson) (Lepidoptera: Pyralidae), a pest of mulberry, was first recorded in Karnataka during 1995. Seasonal occurrence of the leaf-roller and its parasitoids, *Phanerotoma noyesi Zettel*, *Apanteles agilis* Ashmead and *A. bisulcata* Cameron (Hymenoptera: Braconidae) was studied for three years from September 1995 to August 1998, near Bangalore, India. The leaf-roller infestation on mulberry begins after commencement of South-West monsoon during June and infestation is severe during North-East monsoon and winter, from October to December, and then declines and tapers off by next April. *P. noyesi* is the most common and widespread parasitoid. *A. agilis* occurred during winter, in the third year of survey. *A. bisulcata* occurred sporadically during rainy seasons.

KEYWORDS: leaf-roller, *Diaphania (Margaronia) pulverulentalis Phanerotoma noyesi*, *Apanteles agilis*, *A. bisulcata*

INTRODUCTION

The leaf-roller of mulberry, *Diaphania* (*Margaronia*) *pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) is a key pest of mulberry in Karnataka. This pest occurred for the first time in 1995 in several areas in the traditional sericulture belt of Karnataka (Geetha Bai *et al.*, 1997). It is spreading to other areas in Karnataka where sericulture is being practiced since recently. It is also reported to have spread to the adjacent states of Tamilnadu and Andhra Pradesh (Rajadurai *et al.*, 1999). This pest has been recorded earlier in Malaysia (Sengupta *et al.*, 1990) and Nagaland, India (Gupta, 1994). *Diaphania* (*Glyphodes*) *pyloalis* is reported from Jammu (Sharma and Tara, 1985), Kashmir (Dar, 1993) and Punjab (Mavi *et al.*, 1996), and also from China (Howard and Buswell, 1925) and Japan (Rangaswami *et al.*, 1976). Survey for *D. pulverulentalis* and its parasitoids was conducted in a selected area, namely, the

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Yerehalli village near Bangalore in Karnataka, for three years and the results are presented here.

MATERIALS AND METHODS

A mulberry plantation of one acre dimension with M_5 variety mulberry was selected for this study at Yerehalli village near Bangalore. This plot was divided into two sub-plots and mulberry leaf for silkworm rearing was utilised alternately by the rearer. One hundred plants, 20 in each of the four corners and 20 in the middle portion, selected for this study were examined for the leaf-roller at monthly intervals, for three years from September 1995 to August 1998. The caterpillars were collected and reared in the laboratory on fresh mulberry leaves. Mortality of the pest caterpillars and pupae, emergence of moths and parasitoids were recorded daily. Meteorological data (rainfall, relative humidity and temperature) were obtained from India Meteorological Department, Meteorological Centre, Bangalore.

RESULTS AND DISCUSSION

Details of leaf-roller infestation of mulberry, parasitism of the pest, rainfall, relative humidity and temperature from September 1995 to August 1998 are shown in Figs 1–3. In September 1995, during rainy season, the infestation was 70% and average number of caterpillars per plant was 1.65. Infestation reached its peak during October and November 1995; all the one hundred plants examined were infested and the average number of caterpillars per plant was 22.30 and 16.60, respectively. With cessation of rains during December, relative humidity and temperature decreased, and leaf-roller infestation declined to 49% and only 1.41 leaf-roller caterpillars per plant were observed. During January and February 1996, when relative humidity further decreased and day temperature increased, leaf-roller infestation declined to 25 and 13%, and average number of caterpillars per plant to 0.43 and 0.16, respectively. Mulberry plantations were free from leaf-roller infestation from March to August 1996, even though pre-monsoons were experienced during April and May 1996 and heavy rains due to South-West monsoons during June and August 1996, with consequent rise in relative humidity and decrease in temperature.

During the first year of occurrence of the pest, two species of parasitoids, *Phanerotoma noyesi* Zettel and *Apanteles bisulcata* Cameron (Hymenoptera: Braconidae) were recorded from *D. pulverulentalis*. Of these *P. noyesi* was found to be predominant. It was first recorded in November 1995, when infestation of mulberry by the leaf-roller was at its peak. During December 1995 parasitism by *P. noyesi* increased to 22% though pest damage had declined to 49% and then parasitism decreased to 17% in January 1996 and did not occur during February 1996, when pest infestation declined to 13%. *A. bisulcata* was recorded only once during the first year of infestation in October 1995 and the parasitism was only 2%.

During the second year of the survey, leaf-roller infestation in the identified mulberry plantation occurred during September 1996 (67%) and increased during

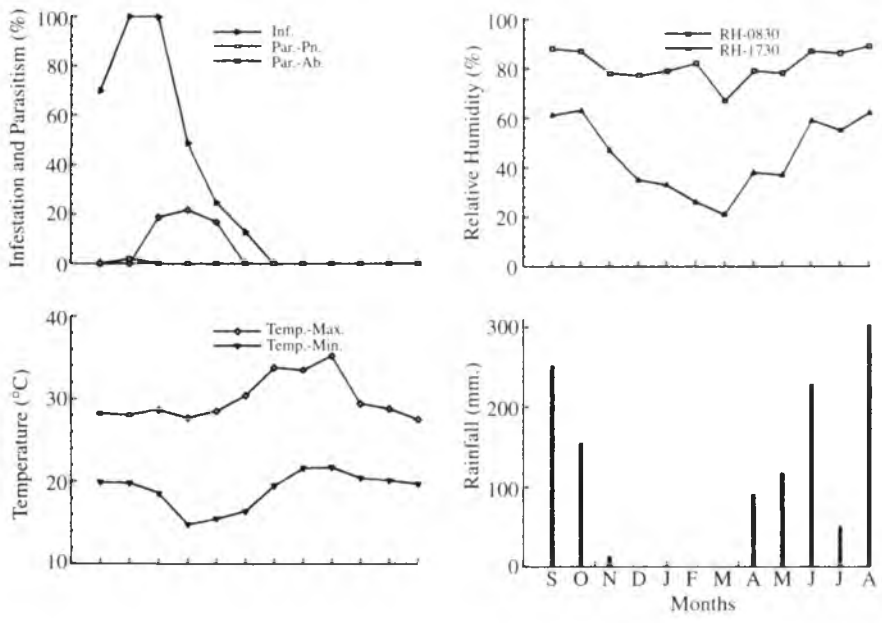

FIGURE 1. Seasonal occurrence of leaf-roller pest and its parasitoids at Yerehalli village during 1995–96 in relation to rainfall, relative humidity and temperature.

October 1996 (78%) and reached its peak (100%) in November 1996 during the North-East monsoons (Fig. 2). Leaf-roller infestation declined to 79, 37 and 5% during December 1996, January and February 1997, respectively. The number of caterpillars per plant was low during the second year of pest occurrence, compared to the first year (Fig. 4). This pest did not infest the mulberry plantation during March to May 1997 in the study area, though light to occasional showers were experienced. Only *P. noyesi* occurred from September 1996 to January 1997 (Fig. 2). Highest parasitism (26.2%) was recorded when the pest population was at its peak (100%) during November 1996.

Leaf-roller was found to infest mulberry plantations for the third consecutive monsoon season during June 1997, when South-West monsoons intensified, and increased gradually, reached its peak (100%) during December 1997 (Figs 2 and 3), and later declined gradually till April 1997. The number of caterpillars per plant was highest (7.49) during December 1997, which coincided with the highest infestation.

Parasitism due to *P. noyesi* was recorded from July 1997 (9.4%). It reached its peak (67.8%) during August 1997, when infestation was 85% and declined to 33.0% during September 1997, with the decline in infestation to 82.0%. Later, it gradually decreased to 14.4, 15.6, 19.2, 19.3 and 10.6%, respectively, from October 1997 to February 1998. *Apanteles agilis* Ashmead (Hymenoptera: Braconidae) was recorded for the first time from *D. pulverulentalis* during December 1997. Parasitism due to *A. agilis* was 11.1

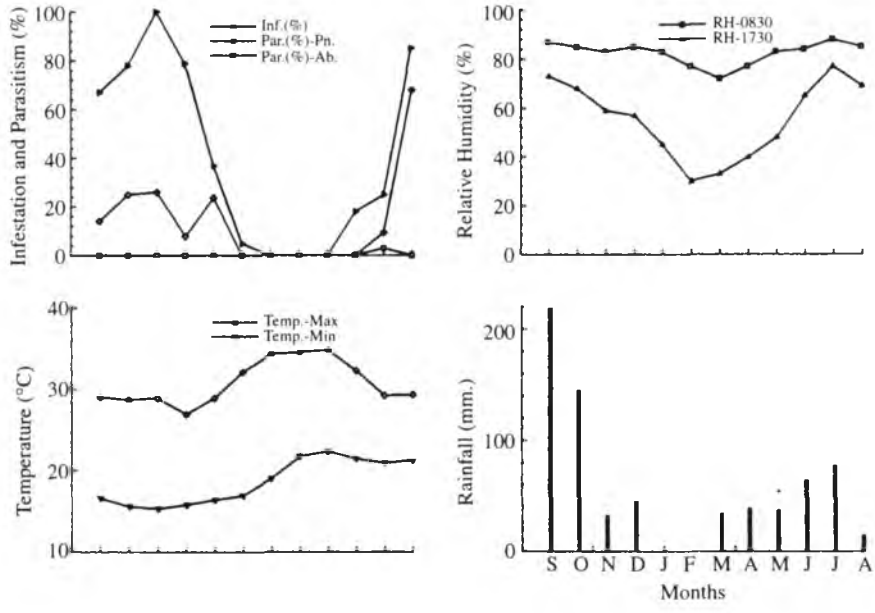

FIGURE 2. Seasonal occurrence of leaf-roller pest and its parasitoids at Yerehalli village during 1996–97 in relation to rainfall, relative humidity and temperature.

and 10.5% during December 1997 and January 1998, respectively. *A. bisulcata* was recorded (3.0%) only once during July 1997.

D. pulverulentalis was found to infest mulberry plantations for the fourth consecutive monsoon season from June 1998 (12.0%) and infestation increased to 50.0 and 60.0% during July and August 1998, respectively, as monsoon intensified. Parasitism due to *P. noyesi* was 4.25 and 16.95% during July and August 1998.

Rajadurai *et al.* (1999) have reported that leaf-roller of mulberry appears during June and persists upto February, with sporadic appearance during March to May in Karnataka, Andhra Pradesh and Tamil Nadu in India. However, Sengupta *et al.* (1990) report that *D. pulverulentalis* attack is usually noticed between the end of rainy season and to the end of dry season in Malaysia.

Damage to mulberry plantations by *D. pulverulentalis* begins around June, after South-West monsoon intensifies. Infestation is severe from October to December, during North-East monsoon and early winter when relative humidity is high and temperature is low. Later, infestation declines and this trend continues till next April, when humidity decreases and temperature rises.

P. noyesi is the most common and important parasitoid of *D. pulverulentalis*, the highest parasitism recorded being 67.8%. It occurs from July to next February–March. Parasitism increases with increase in the host population and declines with that of the host. *A. bisulcata* occurred occasionally and sporadically in October 1995 and

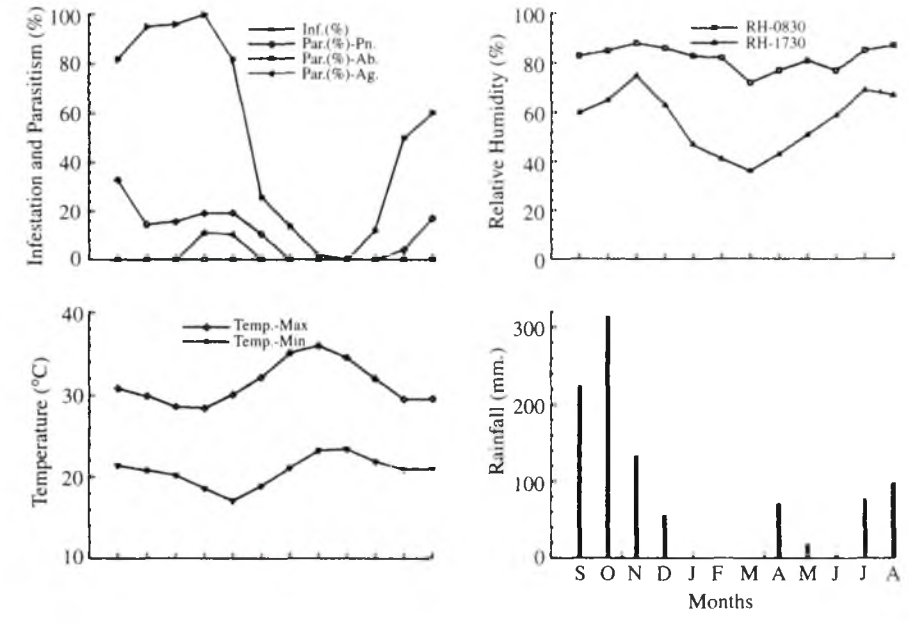

FIGURE 3. Seasonal occurrence of leaf-roller pest and its parasitoids at Yerehalli village during 1997–98 in relation to rainfall, relative humidity and temperature.

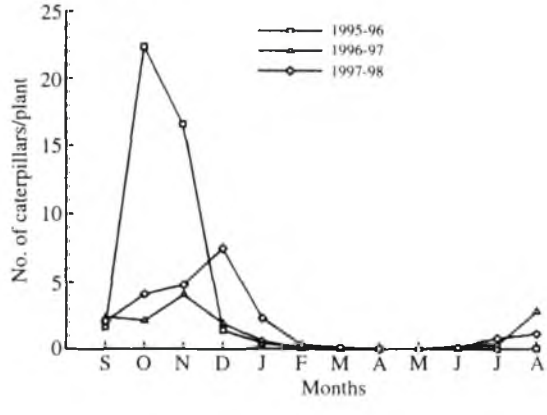

FIGURE 4. No. of caterpillars per mulberry plant recorded in different periods.

July 1997, during rainy season. *A agilis* occurred during December and January 1998 in winter, during the third year of the survey.

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A comparative study on the trehalose level in different varieties of the silkworm, *Bomyx mori*, during fifth instar larval development

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ABSTRACT: The level of Trehalose in the haemolymph of fifth instar larva of three different varieties of the silkworm viz., Biovoltine (BV), Multivoltine (MV) and the cross bread (CB) was investigated. The trehalose level was the least on the first day in all the three varieties which increased with the development of fifth instar larva, attained the peak on the sixth day in case of BV and CB, on the seventh day in case of MV and decreased thereafter till spinning. The trehalose level was found to be maximum in BV followed by CB and MV. Interestingly the blood trehalose level was shown to be maintained at a more or less constant level during the active feeding period, i.e. from fourth day to sixth day, of the fifth instar development in all the three varieties. The results are discussed in relation to the food consumption and the regulation of trehalose level in the silkworm larva.

KEYWORDS: *Bomyx mori*, bivoltine, multivoltine, crossbread, trehalose

INTRODUCTION

Insects need carbohydrates as major fuel for their growth and development, being derived, mostly from the diet. The silkworm, *Bmobyx mori*, conserve sufficient quantity of energy reserve during larval stage, to be utilized during pupal and adult stages. Trehalose is the major and metabolically active, nonreducing disaccharide in the insect blood (Wyatt and Kalf, 1956, 1957) which is synthesized in the fat body, (Candy and Kilby, 1959, 1961; Clegg and Evans, 1961) and utilized during spinning, flight and starvation of insects (Saito, 1960; Horie, 1961). It is well known that haemolymph, the only extracellular fluid in insect, is having diverse functions (Pawar and Ramakrishnan, 1977). It is the reservoir for most of the biochemicals that are required for nearly every physiological activity of the insect. Thus the change in the composition of haemolymph reflects the morphogenic and biochemical changes

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taking place in insect tissues. The insect haemolymph performs several physiological functions such as immunity, transport, storage etc. (Mullins, 1985). In the present investigation an attempt has been made to study the changes in trehalose level in the haemolymph during fifth instar development of different varieties of the silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

Disease free layings of three different silkworm varieties viz., Bivoltine (NBD 42), Multivoltine (Pure Mysore PM) and the cross bread (PM × MBD42) were obtained from NSSP Bangalore, and the eggs were incubated at a temperature of 25°C and a relative humidity of 75%. After hatching, the larvae were reared under standard laboratory conditions on mulberry leaves (M5 variety). During young age rearing (up to III instar), the worms were maintained at a temperature of 26–28°C and a relative humidity of 80%–90%. Whereas during late age (rearing IV and V instar), the worms were maintained at a temperature of 24–26 °C and a relative humidity of 70%–80% as recommended by Krishnaswamy *et al.* (1973). The fifth instar larvae were used for the experiment. The haemolymph was collected from the larvae in a clean prechilled test tube containing a few crystals of thiourea, by cutting the caudal horn (Kuwana, 1937). The haemolymph was centrifuged at 3000 rpm for 10 min. The supernatant was collected and used for the estimation of trehalose (blood sugar) according to the procedure of Saito (1960), spectrophotometrically by phenol sulphuric acid method (Dubotis *et al.*, 1956) using glucose as standard. Four to five worms were used for each data point. The mean value along with the standard dedication is reported in the results.

RESULTS

The haemolymph trehalose level was found to differ in all the three varieties during fifth instar larval development. It was found to be 3.60 mg ml in BV, 3.60 mg/ml in CB and 3.45 mg/ml in MV on the first day. Thereafter a significant increase in the level was observed with advancement of age, to reach the maximum on the sixth day in BV (8.73 mg/ml) and CB (8.69 mg/ml), while on the seventh day in case of MV 5.98 mg/ml). The trehalose level decreased on the final day (spinning day) of fifth instar larval development. When compared between the varieties, the concentration of trehalose was maximum in BV (8.73 mg/ml) followed by CB (8.69 mg/ml) and MV (5.98 mg/ml) (Table I, Fig. 1).

DISCUSSION

Trehalose is the major disaccharide in the insect haemolymph (Wyatt and Kalf, 1956, 1957). It's active bio synthesis in the fat body (Candy and Kilby, 1959, 1961; Clegg and Evans, 1961) and rapid utilization during flight and starvation of insects (Saito, 1960; Horie, 1961) has been reported clearly. The level of trehalose in the haemolymph

TABLE 1. Changes in the level of trehalose (blood sugar, mg.ml) in the haemolymph of V instar larva of the silkworm, *Bomyx mori*. L

Duration in days	Silkworm varieties		
	Bivoltine (NB4D2)	Multivoltine (PM)	Cross breed (CB)
1.	3.788 ± 0.099	3.450 ± 0.000	3.602 ± 0.148
2.	4.600 ± 0.000	3.680 ± 0.163	3.912 ± 0.130
3.	7.134 ± 0.698	3.910 ± 0.000	5.104 ± 0.190
4.	7.912 ± 0.126	4.830 ± 0.000	6.020 ± 0.247
5.	8.280 ± 0.000	5.290 ± 0.163	8.082 ± 0.072
6.	8.726 ± 0.139	5.566 ± 0.103	8.694 ± 0.192
7.	8.280 ± 0.000	5.980 ± 0.000	7.222 ± 0.126
8.	7.456 ± 0.148	5.340 ± 0.130	6.892 ± 0.102
	CD-1 @ 5% = 0.8955		
	@ 1%=1.2555		
	CD-2 @ 5% = 1.3678		
	@ 1% = 1.9178		

CD-1: Comparison between silkworm varieties; CD-2: Comparison between days.

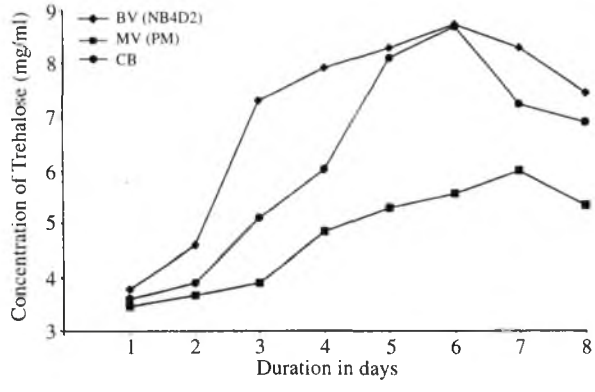

FIGURE 1. Changes in the level of Trehalose (Blood sugar) (mg/ml) in the haemolymph of the fifth instar larva of silkworm *Bombyx mori*.

reflects the level of carbohydrates in the body was well as the physiological status of the insect. The level of trehalose is found to be the lowest on the first day of fifth instar in all the three varieties. This may be due to the fact that the tender mulberry leaves fed to the 'out-of-moult' worms contain more proteins and less sugars leading to the lowered consumption of carbohydrates (Krishnaswamy *et al.*, 1973; Benchamin and Nagaraj, 1987). The increase in the trehalose level with the development of fifth instar larva perhaps be due to feeding of coarse leaves, which are rich in carbohydrates and

as well as the higher rate of food consumption by the larva resulting in increased absorption of glucose through the digestive tract (Horie, 1959) to cope with the increased physiological activities. It has been reported that the haemolymph trehalose level in insects is maintained by the absorption of digested sugars through the gut (Horie, 1959) and /or by the break down of fat body glycogen to glucose which serve as the precursor for the synthesis of trehalose (Candy and Kilby, 1959, 1961; James *et al.*, 1961). Clegg and Evans (1961) reported the active biosynthesis of trehalose in the fat body which are released in to the haemolymph. Thus the peak level of trehalose observed on the sixth day in BV and CB and on the seventh day in MV may be associated with active depletion of fat body glycogen along with the increased absorption of sugars through the gut.

The decrease in the level of trehalose on the final day of fifth instar is the result of its greater utilization to furnish fuel for active synthesis sof silk and other physiological processes like spinning (Evans and Dethier, 1957; Bucher and Klingenberg, 1968; Clegg and Evans, 1961). Further it may also be associated with the decreased food intake during the latter part of the fifth instar.

Among the varieties studied, the trehalose level is found to be high in BV followed by CB and MV. This may perhaps be due to higher physiological activities such as food intake, digestion, assimilation and conversion in BV compared to CB and MV (Gururaj, 1995). Further, the level of trehalose is found to be maintained at more or less a constant level between fourth and sixth day of fifth instar development in BV and MV where as in case of the CB, the level was maintained between fifth and sixth day, there by maintaining the homeostasis (Saito, 1960, 1963) probably at the expense of fat body glycogen. Thus the present results are in accordance with the earlier finding of Saito (1963) suggesting the existence of a possible homeostasis mechanism in the silkworm to regulate the trehalose level in the body.

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Biology, adult feeding, oviposition preference and seasonal incidence of *Othreis materna* (Linnaeus) (Lepidoptera: Noctuidae)

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ABSTRACT: *Othreis materna* (L.) carried out its biological activity for ten months (April to January) on irrigated *Tinospora cordifolia* Miers (Menispermaceae) at GKVK Campus, University of Agricultural Sciences, Bangalore. At other places, where *T. cordifolia* was growing wild, larvae were observed only in September–October. The larvae of *O. materna* could feed and complete development only on the species of the genus *Tinospora* (*T. cordifolia* and *T. sinensis* (Lour.) Merr.).

At 25±1° C the incubation period was 3.5–4.0 days. *Othreis materna* generally had five, and rarely six instars. The duration of the larval instars was 13.08 ± 0.73 days. The pupal period varied from 12.5 to 14.0 days. The length and width of grown up larva were 75 mm and 10 mm, respectively. First instar of *O. materna* was light green, the second instar was black and the third and later instars were clay brown with dorsal and subdorsal black lines in monsoon population and brownish black or velvety black without the dorsal and subdorsal lines in post-monsoon population. On an average the larvae with five instars ate 12.0–18.19 g of leaf and produced 5.17–8.57 g excreta and the larvae with six instars ate 15.34–18.19 g of leaf and produced 7.13–8.35 g excreta.

Freshly emerged moths of *O. materna* preferred to feed on tomato (9.3 ± 5.44 feeding holes), followed by banana (3.6 ± 1.96 feeding holes) for the first ten days of exposure. The adult feeding preference in descending order during 1998 was tomato > banana > guava > brinjal > pomegranate > orange > mosambi. The descending order of adult feeding preference during 1999 was guava > tomato > banana > pomegranate > orange > mosambi > brinjal. In captivity, females did not lay eggs on vines of *T. cordifolia*, but oviposited on the nylon net and other surfaces including the fruits on which they were feeding. However, in the field, the eggs were laid on the leaves of *T. cordifolia* and on twigs of plants supporting the vines.

KEYWORDS: Biology, adult feeding, oviposition, *Othreis materna*

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INTRODUCTION

The fruit piercing moth, *Othreis materna* (L.), is one of the serious pests of citrus and pomegranate and occurs along with *O. fullonia* (Clerck), *O. homaena* Hübner, and *O. cajeta* (Cram.) in India (Susainathan, 1924a,b; Ayyar, 1944). Though Srivastava and Bogawat (1968) and Lolage and Khaire (1998) studied the biology of *O. materna* on *Tinospora cordifolia*, the response of its larvae to other Menispermaceae plants was not studied. Information about seasonal occurrence, adult food and ovipositional preference is insufficient. Studies were conducted on biology, adult preference for feeding and oviposition and seasonal incidence of this pest between October 1997 and December, 1999 at College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore and the results are presented in this paper.

MATERIALS AND METHODS

Biology of *O. materna*

Biology of *O. materna* was studied at 25 ± 1 °C on *T. cordifolia* in a BOD incubator (Newtronic model NEC-110-RIS 12 d:12 1). A set of twenty-five neonate larvae was reared individually on *T. cordifolia*. The neonate larva was reared in 100 ml transparent plastic containers up to third instar, later shifted to 250 ml transparent plastic containers (as 100 ml container could not provide sufficient space for grown up caterpillar) till adult emergence. A portion of the lid of these plastic containers was cut and replaced with copper mesh (100 mesh) for aeration. The young larva was provided with tender leaves and the grown up ones with matured leaves. The weight of fresh leaf, fed portion of leaf, caterpillar and excreta were recorded at 24 h interval (Scientech model SA 120, 0.1 mg sensitivity). Moulting was observed at 12 hourly interval and the width of the head capsule was measured. The length and greatest breadth of the caterpillars were recorded at 24 h interval. Pupal weight was recorded one day after pupation. Adult weight was recorded 4 h after emergence.

The pupae were sexed using genital and anal slits and the data on duration, body dimensions, weight and width of head capsule for male and female insect were recorded separately. Data on life stages of male and female on each host were subjected to student 't' test. Data on life stages of male and female on different hosts were subjected to analysis of variance (ANOVA) with unequal replications.

The last instar larvae of *O. materna* were also collected from the field during different months and the larvae reared in the laboratory were utilised for studying the larval polymorphism. (Unless otherwise stated the colour markings of one side of the body described.)

Larval host specificity

Twenty-five neonate larvae of *O. materna* were provided with tender leaves of each of the available Menispermaceae in separate plastic containers (250 ml). The petiole of the leaf was dipped in a vial containing water to avoid desiccation. The species on which most of the released larvae survived to second instar was considered as a natural

host. The Menispermaceae not fed by the neonate larvae were considered as non-hosts and were again tested by smearing leaf extract of non-host plants on egg-chorion, which neonate larvae often fed. For carrying out these studies a batch of twenty five eggs of *O. materna* was glued to a white paper card and smeared with aqueous leaf extract (prepared by crushing 10 g leaf in 50 ml of water) of non-host of *O. materna*, and shade dried and labelled. After the larvae had consumed the egg chorion, tender leaves of the Menispermaceae used for smearing the eggs were provided. Whenever the larvae died, the host plant was re-confirmed as a non-host. Further older larvae (a batch of ten newly moulted lalrvae of II, III, IV and V instar) reared on *T. cordifolia*, were offered foliage of non-host plants.

Adult food and oviposition preference

Field surveys were conducted in and around Bangalore, Dharwad, Mudigere, Raichur and Bijapur. Different species of Menispermaceae naturally growing in and around GKVK Campus, Bangalore were surveyed at fortnightly interval. *Tinospora cordifolia* Miers, *Cocculus hirsutus* Diels, *Anamirta cocculus* W. & A., *Stephania japonica* Miers, *Stephania wightii* Dunn., *Cyclia peltata* Diels and *Diploclisia glaucescens* Diels were visited once in fifteen days and all the eggs and larvae found on these vines were collected and reared in the laboratory for determination of *Othreis* species breeding on them. The survey also helped in the identification of natural larval host plants of *O. materna*.

Caterpillars of *O. materna* were collected around Bangalore on *T. cordifolia*. The larvae were reared with leaves of their host plants in a cage (0.3 × 0.3 × 0.3 m.) provided with aeration on three sides. Adults that emerged were enclosed in nylon mosquito nets of size 2 × 2 × 3 m. Ripened fruits such as guava or banana were hung in the cage to serve as adult food. Eggs obtained from these moths were used to study the biology.

RESULTS AND DISCUSSION

Biology of *Othreis materna* (L.)

At 25 ± 1 ° C, *O. materna* completed development from egg to adult stage on *T. cordifolia* in 32.42 to 34.25 days with majority of larvae undergoing five larval instars (Table 2). Various biological parameters of stages of development are presented below.

Egg

Egg was hemispherical with a flat bottom and measured 0.86–0.94 mm in diameter (Table 1) and never crossed 1 mm width, whereas Srivastava and Bogawat (1968) recorded egg width of 0.95–1.05 mm at Udaipur. When freshly laid it was creamy white and turned light yellow with four brown markings (indicating developing mandibles) before hatching. The chorion was thick and hard. Eggs were laid singly on dorsal or ventral surface of leaf. On the leaves of cultivated *T. cordifolia*, out of 1001

TABLE 1. Body dimensions of life stages of *Othreis materna* (L.) reared on *Tinospora cordifolia* (in mm)

Stage	Male			Female		
	Length	Width	Head capsule width	Length	Width	Head capsule width
Egg		0.86–0.94(0.92 ± 0.02)				
Larvae with five instars[a]						
Instar I	6.5–8.0 (7.17 ± 0.61)	0.6–0.9 (0.76 ± 0.10)	0.84–0.98 (0.92 ± 0.05)	5.5–8.0 (7.07 ± 0.78)	0.7–0.8 (0.76 ± 0.05)	0.49–0.51 (0.50 ± 0.01)
II	11-14 (12.08 ± 0.83)	1.0–1.2 (1.12 ± 0.07)	1.46–1.63 (1.52 ± 0.05)	11-14 (12.43 ± 0.90)	1.0–1.2 (1.11 ± 10.08)	0.90–0.96 (0.92 ± 0.02)
III	20–27 (22.38 ± 2.06)	1.7–2.5 (2.20 ± 0.30)	1.46–1.63 (1.52 ± 0.05)	19–23 (21.0 ± 1.51)	1.7–2.5 (2.24 ± 0.31)	1.43–1.59 (1.51 ± 0.06)
IV	35–47 (38.92 ± 3.22)	3.5–5.0 (4.08 ± 0.38)	2.33–2.60 (2.53 ± 0.79)	35–44 (39.71 ± 2.66)	3.5–5.0 (4.21 ± 0.45)	2.33-2.73 (2.53 ± 0.13)
V	60–75 (67.54 ± 4.01)	7.0–10.0 (8.54 ± 0.82)	4.0–4.2 (4.05 ± 0.07)	66.0–74.0 (71.0 ± 2.45)	9.0–10 (9.07 ± 0.56)	3.8–4.3 (4.08 ± 0.17)
Pupa	28–33 (30.31 ± 1.44)		(9.52–11 (9.92 ± 0.58)	28–31 (29.71 ± 0.88)	9.5–11 (10.14 ± 0.58)	
Larvae with six instars[b]						
Instar I	7.0–8.0 (7.50 ± 0.50)	0.72–0.9 (0.80 ± 0.10)	0.51 (0.51 ± 0.00)	8.00	0.90	0.51
II	9–11.0 (10.00 ± 1.00)	0.92–1.2 (1.05 ± 0.15)	0.86–0.90 (0.88 ± 0.02)	12.00	1.10	0.94
III	15–19 (17.00 ± 2.00)	1.9–2.0 (1.95 ± 0.05)	1.23–1.33 (1.28 ± 0.05)	18.00	1.80	1.40
IV	25–30 (27.50 ± 2.50)	3.0–3.5 (3.25 ± 0.25)	1.93–2.13 (2.03 ± 0.10)	27.00	2.50	2.00
V	44–48 (46.0 ± 2.0)	5.00 (5.00 ± 0.00)	2.94 (2.94 ± 0.00)	37.00	4.50	2.93
VI	72–74 (73.0 ± 1.0)	9.00–10.0 (9.50 ± 0.50)	4.30 (4.30 ± 0.00)	74.00	9.00	4.50
Pupa	28–30 (29.00 ± 1.00)	9.0 (9.00 ± 0.00)		30.00	9.50	

[a] 13 males; 7 females; [b] 2 males; 1 female.

TABLE 2. Duration of life stages of *Othreis materna* (L.) reared on *Tinospora cordifolia*

Stage	Duration (in days)			
	Male		Female	
	Mean ± SD	Range	Mean ± SD	Range
Egg	3.52 ± 0.10	3.5–4.0		
Larvae with 5 instars				
Number reared	13		7	
Larva I	3.19 ± 0.37	3.0–4.0	2.93 ± 0.17	
II	1.81 ± 0.24	1.5–2.0	1.93 ± 0.17	1.5–2.0
III	2.23 ± 0.25	2.0–2.5	2.14 ± 0.23	2.0–2.5
IV	2.54 ± 0.49	2.0–3.0	2.93 ± 0.17	2.5–3.0
V	3.31 ± 0.46	3.0–4.0	4.00 ± 0.00	
Total larval duration	13.08 ± 0.73	12–14	13.93 ± 0.17	13.5–14
Pupa	13.42 ± 0.55	12.5–14	12.93 ± 0.17	12.5–13
Total developmental Period	32 ± 0.88	30–33	32.36 ± 0.23	32–32.5
Larvae with 6 instars				
Number reared	2		1	
Larva I	3.00 ± 0.00	–	3.00	
II	2.00 ± 0.00	–	2.00	
III	2.25 ± 0.25	2.0–2.5	2.00	
IV	2.00 ± 0.00	–	2.00	
V	2.50 ± 0.50	2.0–3.0	2.00	
VI	4.00 ± 0.00	–	4.00	
Total larval duration	15.75 ± 0.75	15–16.5	15.00	
Pupa	13.00 ± 0.00	–	12.50	
Total developmental Period	34.25 ± 0.75	33.5–35	33.0	

eggs collected, 90.1% were laid on the dorsal side thus showing preference to the dorsal side. Incubation period varied from 3.5 to 4 days and was in agreement with the observations of Sontakay (1944), Cherian and Sundaram (1936) and Srivastava and Bogawat (1968). However Lolage and Khaire (1998) recorded only 2.5 days of incubation period and Ayyar (1944) recorded an incubation period of longer duration (8–10 days). This may be due to variation in the temperature under which the observations were made by these workers.

Larva

Generally there were five and rarely six larval instars. Out of 25 neonate larvae reared, 20 larvae had five instars (80%) and only 3 had six instars (12%). The number of

instars observed was independent of sex as five and six instars were found in both the sexes. Of the 20 larvae having five instars, 13 were males and 7 were females. Similarly from 3 larvae with six instars, 2 were males and 1 was female. There was 8% mortality in larval stage. Srivastava and Bogawat (1968) reported five larval instars and Lolage and Khaire (1998) recorded six instars.

Though the larval colour in *O. materna* varied with respect to season the first two instars were similar. The first instar was pale green with brown spots (indicating the bases of brownish setae), integument was transparent through which the gut contents were visible. A few black hairs were present on the body. The general body colour in second instar was black. However, third instar onwards two larval morphs were observed, one in monsoon and the other in post-monsoon population.

Monsoon population

(July–September): The general body colour of third to fifth instar caterpillars was dull clay-brown with dorsal and subdorsal light black bands. Subdorsal bands were intercepted by the eye spot. The general body colour resembled the colour of the stem of *T. cordifolia* and it was difficult to locate the caterpillar resting on the stem. Spiracles of a segment were connected by a row of black spots which appeared like a black band. One-third of the rim encircling the eye spot towards antero-dorsal side was white lined brick red and this portion was replaced by a uniform yellow rim in post-monsoon population. The remaining portion of the rim was black, lined with white in monsoon population and uniform bright orange in post-monsoon population. In both the seasons the centre of the eye spot was of the body colour.

Post-monsoon population

(October–January): The general body colour in third to fifth instars was brilliant black or velvety black with bright blue, white and orange spots. Orange spots were larger and prominent on thoracic segments whereas larger white patches were distinct on segments forming the hump. Spiracles were joined by small white dots.

The colour morphs of *O. materna* observed in the present investigation agree with the reports of Sevastopulo (1940) who correctly described the different colour morphs. The velvety blue form observed by Ayyar (1944) was not observed during the present investigation.

Unless otherwise mentioned the colour pattern of only one side of the body segments of six instars of black form found in post-monsoon season is described hereunder.

First instar

Body yellowish green, head capsule light brown, prothorax with a prominent brownish black patch dorsally and two small patches laterally. Abdomen had ten segments and the segments 4–6 and 10 had prolegs. Body had a few short black setae. Neonate larva was very active, restless and searched the tender leaves travelling long distances (sometimes more than 6 metres). The anterior portion of the body was looped

concealing the head inside and the posterior half of the body was held erect at rest. The duration of this instar varied from 3 to 4 days (Table 2).

Second instar

The larva was greenish brown when freshly moulted and turned brownish black after a few minutes with brownish black head. Meso- and metathorax each had four light blue spots on the dorsum forming an indistinct transverse band antero-dorsally. Abdominal segments 1–3 each with two orange spots on the dorsum and each orange spot encircled by three to four white patches; segments 1–7 each with four light blue patches dorso-laterally; dorsum of segment eight (which formed the hump) with four (two small and two large) white spots, arranged transversely in a row giving the appearance of a white transverse band (These spots are absent in *O. fullonia* and *O. homaena*). The duration of this instar varied from 1.5 to 2 days (Table 2).

Third instar

Body and head were velvety black, thoracic and abdominal spots were as in second instar. The orange spots on abdominal segments 1–3 and the white spots on segment eight were more prominent in this instar. The duration of this instar lasted for 2–2.5 days (Table 2).

Fourth instar

Head was black and body velvety black. There was one brick red or orange spot on each of meso- and metathoracic and first abdominal segments, arranged in a longitudinal line. Abdominal segments II and III each had a prominent eye-spot with a central irregular patch of the body colour; a yellow rim on antero-dorsal periphery and the rest of the circular periphery orange-red with a blue arch at the lower side of the central patch. Segments 7–9 had a yellowish white streak laterally. A dark brown line traversed the body from the metathoracic leg to first pair of prolegs. The duration of this instar was 2–3 days (Table 2).

Fifth instar

Colour was similar to that of the fourth instar. Prothorax had two prominent orange spots on either side of anterior of mid dorsal line, a few small deep blue spots posterior to the latter. Meso- and metathoracic segments had three and two orange spots laterally, respectively, in addition to a distinct row of four blue spots postero-dorsally. Eye spot one each on second and third abdominal segment, had a small yellow patch at antero-dorsal periphery, rest of the circular periphery was orange-red enclosing body coloured area with a thin deep blue arch at the lower end. There were small yellowish or whitish patches from posterior eye-spot to segment seven. Segment eight had a hump and three white patches dorso-laterally. The duration of this instar varied from 3 to 4 days in population with five instars and 2–3 days in population with six instars (Table 2).

Sixth instar

Very similar to fifth instar in all respects. The duration of this instar was 4 days (Table 2). The total larval duration ranged from 12–14 days in larvae undergoing five instars and 15–16.5 days in larvae undergoing six instars (Table 2). The duration of second, third and fourth instars recorded in the present investigation was in accordance with the observations made by Lolage and Khaire (1998) except that the duration of the first and sixth instars was 3–4 and 4 days, respectively but Lolage and Khaire (1998) recorded it as only 2 days in both the instars. Slight variations in the duration of larval instars recorded by Srivastava and Bogawat (1968) may be due to changes in the climatic condition under which the studies were made by them. The weight of grown up caterpillar varied from 3.33 to 3.894 g, but Lolage and Khaire (1998) recorded a lower larval weight of only 2.3 g. Hargreaves (1936) reported total larval and pupal periods of *O. materna* as 16–17 and 11–12 days, respectively, when reared on *Rhigicarya racemifera* Miers in Sierra Leone.

The head capsule width of first, second, third, fourth, and fifth instar larva was 0.5, 0,9 1.5, 2.5 and 4.0 mm, respectively in the present study. However, Srivastava and Bogawat (1968) recorded much higher width i.e., 0.8, 1,2, 1.8, 2.7, and 4.2 mm, respectively. Considering the larger size of egg and larvae observed by Srivastava and Bogawat (1968), it seems that there may be geographical size variation in *O. materna* which needs to be investigated.

Total quantity of leaf consumed and excreta produced ranged from 12–18.19 g and 5.17–8.57 g in larvae undergoing five instars and 15.35–18.19 g and 7.13–8.35 g, in larvae undergoing six instars, respectively (Table 3).

Pupa

Pupation took place in a thin cocoon of white silk spun between leaves. The pupa was dark brown, shiny and punctate. Anterior end of the pupa was blunt while the posterior was conical. Wing pads were distinct and darker than body. The intersegmental area of the abdomen was darker. The spiracles were black and visible on prothorax and abdomen segments II–VIII. The apex of the abdomen had the cuticle wrinkled into a honey comb pattern and the cremaster consisted of hooked spines on either side. The pupal duration was 12.5–14.0 days in both sexes (Table 2). The duration of pupa recorded in the present investigation is in accordance with the observations of the earlier workers. The minor variations observed in the life parameters between the present investigation and that of Lolage and Khaire (1998) and Srivastava and Bogawat (1968) may be due to change in the climatic conditions at different places where the insect was reared.

Preference of the moth for food and oviposition

The feeding punctures were maximum on tomato (9.3 ± 5.44), followed by banana (3.6 ± 1.96) for the first ten days of exposure (Table 4). The moths preferred guava (10 ± 2.16 feeding holes) when tomato and banana were withdrawn. In the absence of these three hosts, they preferred brinjal (9.6 ± 6.98 feeding holes). The adult feeding

TABLE 3. Weight of leaf consumed, excreta produced and weight of life stages of *Othreis materna* (L.) reared on *T. cordifolia*

Stage	Weight (in g)			
	Male		Female	
	Mean ± SD	Range	Mean ± SD	Range
Larvae with 5 instars				
Number reared	13		7	
Larva				
I	0.003 ± 0.001	0.002–0.003	0.002 ± 0.001	0.002–0.003
II	0.011 ± 0.001	0.012–0.014	0.012 ± 0.002	0.001–0.014
III	0.071 ± 0.015	0.052–0.095	0.069 ± 0.013	0.052–0.089
IV	0.499 ± 0.079	0.376–0.660	0.537 ± 0.101	0.403–0.671
V	3.639 ± 0.421	2.961–4.411	3.839 ± 0.367	3.163–4.356
Pupa	1.965 ± 0.173	1.625–2.226	1.936 ± 0.174	1.619–2.173
Total leaf Consumed	14.00 ± 1.416	12.000–18.194	14.153 ± 1.425	12.398–16.128
Total excreta produced	6.516 ± 1.060	5.179–8.578	6.072 ± 0.898	5.067–7.352
Larvae with 6 instars				
Number reared	2		1	
Larva				
I	0.002 ± 0.001	0.002–0.003	0.003	
II	0.006 ± 0.001	0.006–0.007	0.009	
III	0.038 ± 0.000		0.034	
IV	0.142 ± 0.001	0.133–0.151	0.140	
V	0.698 ± 0.023	0.675–0.722	0.707	
VI	3.336 ± 0.138	3.198–3.474	3.894	
Pupa	1.771 ± 0.167	1.604–1.938	1.878	
Total leaf consumed	16.771 ± 1.423	15.348–18.194	15.385	
Total excreta produced	7.744 ± 0.610	7.135–8.354	6.648	

preference in descending order during 1998 was tomato > banana > guava > brinjal > pomegranate > orange > mosambi.

Similar studies during 1999 revealed slightly different results. Guava was the most preferred fruit with 32.33 ± 20.88 feeding holes, followed by tomato with 13 ± 3.55 feeding holes during the first three days after moth emergence (Table 4). The moths preferred tomato (14.67 ± 1.25 feeding holes), followed by banana (10.67 ± 1.69 feeding holes) when guava was withdrawn 4 days after their emergence. When both guava and tomato were withdrawn seven days after their emergence, the feeding preference of moths changed in favour of banana with 10.33 ± 1.69 feeding holes. The descending order of feeding preference during 1999 was guava > tomato > banana

TABLE 4. Adult feeding preference of *Othreis materna* (L.)

Number of feeding holes (mean ± SD) and preference index						
Banana	Brinjal	Guava	Mosambi	Orange	Pomegranate	Tomato
During 1998						
3.6 ± 1.9*	0.3 ± 0.6	0.8 ± 1.3	0	0.14 ± 1.2	0.3 ± 0.9	9.3 ± 5.4
38.7[1]	3.2	8.6	0	4.3	3.2	100
	1 ± 0.8	10 ± 2.2	0	0	1 ± 0.8	
	10[2]	100	0	0	10	
	9 ± 7.00		0.3 ± 0.5	2.3 ± 0.5		
	100[2]		3.7	3.7	25.92	
During 1999						
7.3 ± 6.6	0.75 ± 0.5	32 ± 20.9	0	0	0	13 ± 3.6
22.7[2]	2.0	100	0	0	0	40.2
10.7 ± 1.6	0		0	0	0	14.7 ± 1.2
72.72[2]	0		0	0	0	100
10.3 ± 1.7	0		0	0	2.33 ± 1.7	
100[2]	0		0	0	22.58	
	0		0	0	14 ± 2.2	
	0		0	0	100	
	2 ± 2.8		3 ± 3.6	10.7 ± 1.5		
	18.75[2]		28.12	100		
	5 ± 2.2		11 ± 1.6			
	45.45[2]		100			

[1] Preference index when observations continued for 10 days in 1998 and for 3 days in 1999 on provided host.
[2] Preference index upon removal of preferred host in sequence after three days of recording observation.

> pomegranate > orange > mosambi > brinjal. The food preference index was the highest for tomato (100), followed by banana (38.7) during 1998 and for guava (100) followed by tomato (40.2) during 1999 (Table 4). But surprisingly maximum feeding was recorded on pomegranate, orange and mosambi in the field by this moth by several earlier workers. This may be because of the availability of only these fruits to the adult moths during September to October.

Ovipositional preference by adults

The moths did not lay eggs on the leaves of any of the potted Menispermaceae and *Erythrina indica*. However, seven days after emergence, the female moth laid 3 eggs on banana, 2 eggs on tomato and 1 egg on pomegranate fruits. On eight and subsequent days after emergence the moths laid more eggs only on the nylon net, the cloth joining

the net at corners and on the iron rods used to support the fruits. Similar observations were recorded during 1999 when the studies were repeated. Probably the ability of neonate larvae under starvation for a couple of days, to search for tender leaves, travelling over 6 meters, might have made the female moths not take care to deposit the eggs on the leaves of the larval host plants under captivity. But under field conditions most of the eggs were laid on the leaves of *T. cordifolia*. Srivastava and Bogawat (1968) mentioned that the female of *O. materna* laid eggs on any convenient substrate.

Maddison (1982) mentioned the eggs of *O. fullonia* were often deposited on the leaves of plants growing near *Erythrina* trees in New Zealand. Fay and Halfpapp (1993) reported that the eggs of *O. fullonia* were not necessarily laid on the larval host plants. Hence they did not choose to sample the eggs on Menispermaceae and recorded only the larvae and pupae in the field study in Australia. In Australia, females of *Eudocima salaminia* (Cramer) deposited the eggs on leaves of the food plant or any other substrate nearby (Sands *et al.*, 1991).

Seasonal incidence

Studies on seasonal incidence of *O. materna* on *T. cordifolia* at the GKVK Campus, Bangalore, indicated that egg laying continued from end April/early May to January. However, oviposition activity was at its peak during October to December in 1997 and 1998 and during September to October 1999. Maximum of larvae were found in November 1997 and in October 1998 and 1999 (Table 5). This was due to the availability of tender foliage as these vines were cultivated under irrigatiion. On naturally growing vines, Sontakay (1944) found that *O. materna* made first appearance by the end of June and first batch of eggs was laid by the first week of July around Nagpur. He reported larval activity on *T. cordifolia* till the middle of October and thereafter larvae were not found even though the creeper remained green. In the present studies the moth activity was observed for almost ten months from April to January on irrigated crop. There was no correlation between egg and larval counts and the temperature, relative humidity (Fig. 1 and 3) and the rainfall. However usually the peak egg and larval counts followed the peaks of rainfall during 1998 and 1999 (Fig. 2 and 4).

Tinospora cordifolia vines grew naturally at Jakkur and Hebbal. Seasonal incidence studies on these vines indicated very sparse population of *O. materna*. Eggs were hardly noticed. Very less number of larvae were observed on these vines. There was no foliage (tender and old) on the vines from December to May. Tender foliage was produced only after rains in June. Maximum numbers of larvae were observed during October 1997, second fortnight of August and first fortnight of September 1998. Very few larvae were observed during 1999. At Hebbal, observations on the seasonal incidence could not be made during 1999 as the vines were removed.

Larval host plants in the field

In Bangalore, the larvae of *O. materna* were found only on the leaves of *T. cordifolia*. The larvae were also observed on *T. cordifolia* at Raichur, Bijapur, Chettalli, Dharwad,

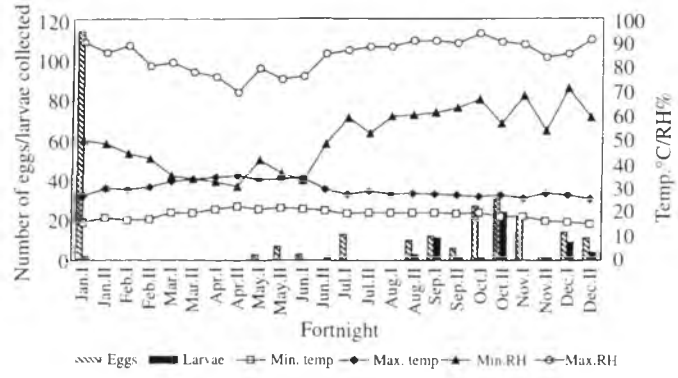

FIGURE 1. Relationship between egg and larval population of *Othreis materna* (Linnaeus) and temperature and relative humidity during 1998.

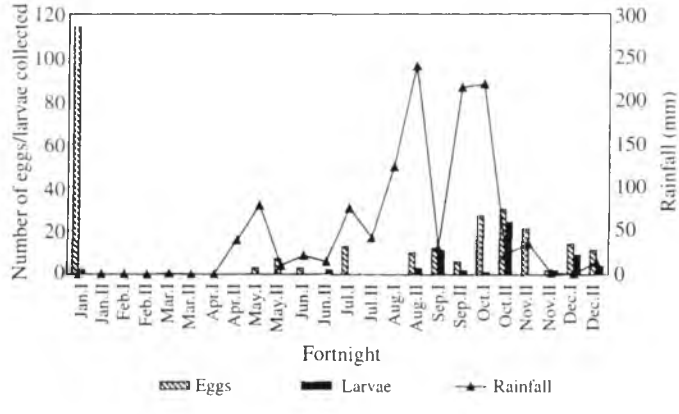

FIGURE 2. Relationship between egg and larval population of *Othreis materna* (Linnaeus) and rainfall duirng 1998.

Pune (Maharashtra) and Tirupati (Andhra Pradesh). *T. cordifolia* was usually found in all the disticts of Karnataka except a few places in Western Ghats. Larger vines of *T. cordifolia* were found climbing on the hedges or on the wires supporting the electric poles adjacent to fruit orchards. During one time survey, larvae of *O. materna* were not naturally observed on *Tinospora sinensis* (Lour.) Merr., which was found in Western Ghats. But under no choice tests, the larvae fed on *T. sinensis*. The results of the present investigation were in agreement with the observations of Susainathan (1924a), Bajpai (1955) and Ayyar (1944), who had reported that the larvae of *O. materna* fed on leaves of *T. cordifolia*.

The larvae of *O. materna* were not observed to feed on the leaves of *Erythrina indica* Lam. Srivastava and Bogawat (1968) also reported that larvae of *O. materna* fed only on *T. cordifolia* and did not survive on *Caesalpinia*, *Cocculus* and *Erythrina*.

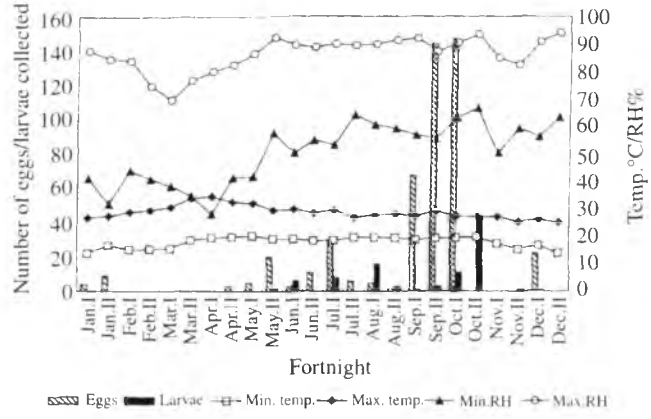

FIGURE 3. Relationship between egg and larval population of *Othreis materna* (Linnaeus) and temperature and relative humidity during 1999.

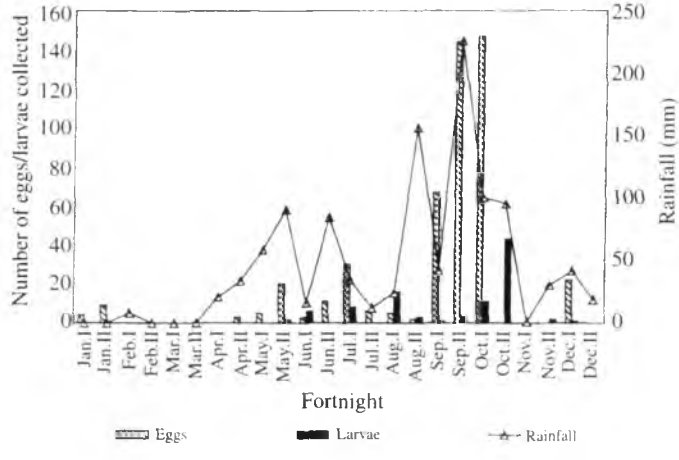

FIGURE 4. Relationship between egg and larval population of *Othreis materna* (Linnaeus) and rainfall during 1999.

In Australia, larvae of *O. materna* fed on *T. smilacina*, another species of *Tinspora* (Fay and Halfpapp, 1993). Larvae refused to feed on *Tiliacora* sp. in Sierra Leone (Hargreaves, 1936). Now it is conclusively confirmed that the larvae of *O. materna* feed only on *T. cordifoia* and also can feed on *T. simensis* in India.

Host specificity of larvae

All the twenty-five neonate larvae could successfully feed and develop normally only on *T. cordifolia* and *T. sinensis*. Although neonate larvae fed on egg chorion smeared with aqueous leaf extract of other Menispermaceae plants, *viz.*, *C. hirsutus*,

TABLE 5. Egg and larval population of *Othreis materna* during 1997–1999 in Bangalore

Month	At UAS, Bangalore# during								$ at Jakkur during					$ at Hebbal during			
	1997		1998		1999				1997	1998	1999			1997	1998		
	E	L	E	L	E	L	F		L	L	L	F		L	L	F	
							T	O				T	O			T	O
Jan. I			114	2	4	0	I	0		0	0	0	I		0	I	I
II			0	0	9	0	I	0		2	0	0	0		0	0	I
Feb. I			0	0	0	0	I	0		0	0	0	0		0	0	0
II			0	0	0	0	I	0		0	0	0	0		0	0	0
Mar. I			0	0	0	0	I	I		0	0	0	0		0	0	0
II			0	0	0	0	I	I		0	0	0	0		0	0	
Apr. I			0	0	0	0	I	I		0	0	0	0		0	0	0
II			0	0	3	0	I	I		0	0	0	0		0	0	0
May I			3	0	5	0	II	II		0	0	I	0		0	I	0
II			7	0	20	2	II	II		0	0	I	0		0	I	I
Jun. I			3	0	3	6	III	II		0	0	I	I		0	I	I
II			0	2	11	0	III	II		0	0	II	I		0	II	I
Jul. I			13	0	30	8	II	III		0	0	II	II		0	II	II
II			0	0	6	0	II	III		0	0	II	II		0	ii	II
Aug. I			0	0	5	16	II	III		0	0	II	II		0	II	II
II			10	3	2	3	III	II		16	0	III	II		0	II	III
Sept. I			12	11	67	1	III	III		15	0	III	III		0	III	III
II			6	2	144	3	III	III		0	0	III	III		1	III	III
Oct. I	0	0	27	1	147	11	II	III	7	0	0	III	III	1	0	III	III
II	30	2	30	24	0	43	II	III	3	3	0	III	III	3	2	III	III
Nov. I	74	9	21	0	0	0	I	II	0	0	0	II	III	3	2	II	III
II	84	9	2	2	0	2	I	I	0	0	1	I	II	0	1	I	III
Dec. I	26	0	14	9	22	1	0	1	0	1	2	I	I	0	1	I	II
II	36	0	11	4	0	0	0	1	0	0	0	I	I	0	0	1	I

I II

grown under irrigation; $ grown under natural condition E–eggs; L–larvae; F–foliage; T-tender; O-Old Foliage grade: 0–no foliage I–less foliage; II–medium foliage; III–heavy foliage. Stages of *O. materna* were collected in *Tinospora cordifolia*

A. cocculus, *S. japonica*, *S. wightii*, *C. peltata* and *D. glaucescens* and a Leguminoceae plant *E. indica*, all the twenty-five larvae died without development into second instar on all other Menispermaceae plants provided except *T. cordifolia* and *T. sinensis*.

The larvae reared to second and third, fourth and fifth instar on *T. cordifolia*, then transferred on to leaves of other Menispermaceae failed to feed on them. This confirmed that larva of *O. materna* is a oligophagous species feeding on species of *Tinospora*.

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Three new species of *Mesopolobus* Westwood (Hymenoptera: Chalcidoidea: Pteromalidae) from India

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ABSTRACT: Three new species of *Mesopolobus viz.*, *M. keralensis*, *M. minutus* and *M. harithus* are described from India. Key to the Indian species of *Mesopolobus* is provided.

KEYWORDS: *Mesopolobus*, New species, Pteromalidae

INTRODUCTION

Mesopolobus Westwood is one of the species rich genera of Pteromalidae (Chalcidoidea) which includes several varied species groups. The genus is world wide in distribution and well known from Europe, North America and other parts of northern temperate zone but little known in the tropics (Graham, 1969; Boucek, 1998). Boucek *et al.* (1979) reported the occurrence of the genus from India and adjacent countries by at least four indetermined species from Pakistan, Nepal and India. In this paper descriptions of three new species of *Mesopolobus* from India and a key to separate them are provided. The type material of the new species are deposited in Zoological Survey of India, Western Ghats Field Research Station, Calicut.

The following abbreviations are used the in the text: F1–F3 – Funicular segments 1 to 3, MV – Marginal vein, OOL – Ocell-ocular distance, SMV – Submarginal vein, STV – Stigmal vein, T1 – First gastral tergite.

Key to the Indian species of *Mesopolobus*

1. Antennae rather long (Fig. 1), combined length of pedicel plus flagellum as long as head width; F1–F3 longer than wide; propodeum (Fig. 3) long, medially 0.6× as long as scutellum, plicae sharp and complete; gaster little shorter than

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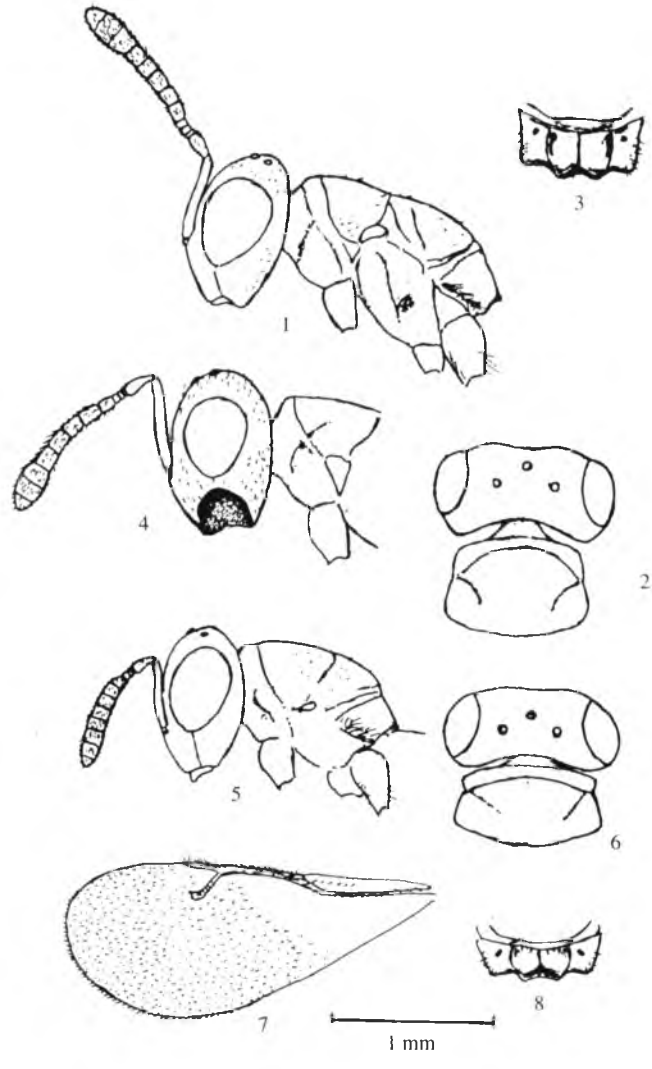
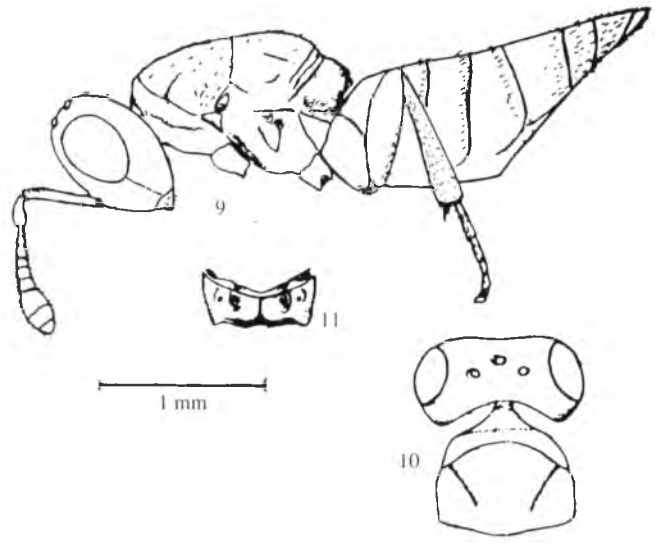


FIGURES 1–4: *Mesopolobus keralensis* sp. nov. Female. 1. Head and mesosoma in profile, 2. Head and mesosoma (part) in dorsal view, 3. propodeum in dorsal view, 4. Male head in profile, 5–8. *Mesopolobus minutus* sp. nov. Female. 5. Head and mesosoma in profile, 6. Head and mesosoma (part) in dorsal view, 7. Fore wing, 8. Propodeum in dorsal view.

head plus mesosoma combined; all legs except coxae yellow .....
..... *M. keralensis* sp. nov.
Antennae short (Figs 5 and 9), combined length of pedicel plus flagellum 0.8–0.9× head width; F1–F3 strongly transverse or quadrate; propodeum short



FIGURES 9–11: *Mesopolobus harithus* sp. nov. Female. 9. Body in profile, 10. Head and mesosoma (part) in dorsal view, 11. Propodeum in dorsal view.

(Figs 8 and 11) medially 0.4–0.5× as long as scutellum, plicae not very sharp as above, complete or incomplete; gaster 1.1–1.2× as long as head plus mesosoma combined; legs with femora pale or dark brown 2

2. Temple broad, length 0.6× eye length; mesoscutum (Fig. 10) width 1.6× its length; pronotal collar anteriorly ecarinate; propodeum medially 0.5× as long as scutellum, plicae not distinct beyond middle; reticulation on head coarse; antennae (Fig. 9) with basal funicular segments strongly transverse; forewing with PMV 0.7× MV; body bright green with golden reflections ..... *M. harithus* sp. nov.
Temple narrow (Fig. 6), length 0.3× eye length; mesoscutum width 2× length; pronotal collar finely carinate anteriorly; propodeum (Fig. 8) medially 0.4× as long as scutellum; plicae fine but complete, reticulation on head finer; basal funicular segments not much transverse; PMV (Fig. 7) short, only 0.5× MV; body bluish black with slight metallic reflection ..... *M. minutus* sp. nov.

***Mesopolobus keralensis* sp. nov (Figs 1–4)**

Female: Length 1.5–2.1 mm (Holotype 1.7 mm). Body bluish green with slight brassy reflection on dorsal part; antennae testaceous; fore and mid coxae brown; hind coxae concolrous with mesosoma, remainder of legs yellow with tips of tarsi brown; tegulae yellow; wings hyaline; veins yellow.

Head: (Figs 1 and 2) moderately and closely reticulate, reticulation finer on lower face and gena. In dorsal view head width 2× length and in front view width 1.2× height; temple length 0.33× eye length; POL 1.7× OOL; malar space length 0.5× eye height; clypeus with anterior margin shallowly emarginate; eyes separated 1.3× their height; eye height 1.3× width. Antennae (Fig. 1) inserted little above lower margin of eyes, scape almost as long as eye (0.9×), hardly reaching median ocellus, basal funicular segments longer than wide; combined length of pedicel plus flagellum equal to head width; clava slightly longer than 3.5 preceding segments combined.

Mesosoma: (Figs 1 and 2) moderately reticulate, reticulation finer on scutellum and axillae; pronotal collar finely carinate anteriorly. Mesoscutum width 1.7× length. Scutellum little convex, medially as long as mesoscutum. Propodeum (Fig. 3) medially 0.6× as long as scutellum, smooth; plicae sharp, complete; spiracles small, oval. Metapleuron almost shiny. Forewing with basal area bare. Relative lengths: SMV 21, MV 12.5, PMV 8.5, STV 6.

Gaster: ovate, little shorter than head plus mesosoma combined (0.9×); in dorsal view length 1.8× width; hypopygium reaching little beyond half length of gaster.

Male: (Fig. 4) length 1.6 mm. Resembles female but differs in having broad depression on gena above mandible and gaster shorter and broader.

Holotype: Female, India, Kerala, Malappuram district, Calicut university campus, 26.v.1988, Coll. P. M. Sureshan.

Paratypes: 1 male, same data as that of holotype except 7.ix.1988, 2 females, Kerala, Malampuzha, 11.xii.1987, 4 females, Kerala, Malappuram district, Parappanangadi, 28.vii.1987; 4 females, 10 males, Calicut university campus, vii-xii.1987, Coll. T. C. Narendran & party; 4 females, Kerala, Parambikulam, 10.xi.1988; 4 females, Kerala, Pathanamthitta district, Konni, 26.xi.1988; 1 female, Calicut district, Madappally, 30.x.1988; 1 female, Trivandrum dist., Akulam, 25.ii.1989; 1 male, Kottayam district, Athirampuzha, 28.xi.1988, coll. P. M. Sureshan.

Remarks: In the key to European species of *Mesopolobus* by Graham (1969) this species keyed out to couplet 48. It resembles *M. prasinus* (Walker) in general morphology and colour but differs from it in having antenna not strongly clavate, combined length of pedicel plus flagellum equal to head width, funicular segments not strongly transverse, pronotal collar finely but distinctly carinate, propodeum medially 0.6× as long as scutellum with median area 1.3× as broad as long. In *prasinus* antenna strongly clavate, combined length of pedicel plus flagellum 0.75–0.85× width of head, funicular segments strongly transverse, pronotal collar not or only indistinctly margined, propodeum medially one third or slightly more than one third of scutellum with median area 1.9–2.1× as broad as long.

***Mesopolobus minutus* sp. nov (Figs 5–8)**

Female: Length 1.1–1.7 mm (Holotype 1.6 mm). Head and mesosoma bluish black with metallic reflection on face; gaster brownish black with bluish reflection on T1 dorsally, antennae testaceous with clava darker; coxae brownish black; femora dark brown with tips and remainder of legs testaceous with tips of tarsi brown; tegulae brown; wings hyaline, veins pale brown.

Head: (Figs 5 and 6) moderately reticulate, clypeus striated, striae extend to genae and area beneath toruli. In dorsal view head with 1.9× length and in front view width 1.2× height; temple narrow, length 0.3× eye length; POL × OOL; anterior margin of clypeus shallowly emarginate; malar space length 0.5× eye height; eyes separated by 1.3× their height; eye height 1.4× width. Antennae with scape little shorter than eye (9 : 10), not reaching median ocellus; pedicel slightly longer than anelli and F1 combined; combined length of pedicel plus flagellum 0.8× head width; clava as long as three preceding segments combined.

Mesosoma: (Figs 5 and 6) moderately reticulate, reticulation finer of scutellum and axillae. Pronotal collar finely carinate anteriorly. Mesoscutum width 2× length. Scutellum medially as s long as mesoscutum, convex. Propodeum medially 0.4× as long as scutellum, shiny, median carina complete, plicae fine but complete; spiracles large, oval. Forewing (Fig. 7) with discal pubescence less distinct, costal cell with few setae on distal end; basal vein and basal cell bare. Relative lengths: SMV 17, MV 9.5, PMV 4.5, STV 4.5.

Gaster: dorsally collapsing, lanceolate, 1.2× as long as head plus mesosoma combined; hypopygium reaching about half length of gaster.

Holotype: Female, India, Kerala, Trichur district, Vazhani, 7.ii.1989, Coll. P. M. Sureshan.

Paratypes: 2 females, data same as that of holotype; 3 females, Kerala Ernakulam, 9.ii.1989, 6 females, Kerala, Konni (Vattamon), 26.ii.1988; 1 female, Kerala, Atingal, 23.ii.1989; 3 females, Kerala, Moolamattom, 30.xi.1988, Coll. T. C. Narendran & party; 1 female, Kerala Eravikulam National park, Turner's hill, 2.iii.1993; 1 female, Kerala, Tellichery, 4.ii.1995, Coll. P. M. Sureshan.

Remarks: In the key to European species of *Mesopolobus* by Graham (1969) this species keyed out to couplet 47 and it resembles *M. nobilis* (Walker) in general morphology. It differs from *M. nobilis* in the colour of head and mesosoma which is bluish black, antennae with pedicel slightly longer than anelli and F1 combined and F1 quadrate (in *nobilis* head and thorax varying from bronze green through green to blue, antennae with pedicel not quiet as long as anelli and F1 combined and F1 subquadrate).

***Mesopolobus harithus* sp. nov (Figs 9–11)**

Female: Length 1.7 mm. Head and mesosoma metallic green with golden reflection; gaster darker; antennae pale brown; legs with coxae concolrous with mesosoma, remainder testaceous with tips of tarsi brown; femora darker; tegulae brown; wings hyaline, veins pale brown.

Head: (Figs 9 and 10) raised reticulate, clypeus striated, striae extend to adjacent areas of face and genae. In dorsal view head with 1.8× length and in front view width 1.2× height; temple vide, length 0.6× eye length; POL 2× OOL; anterior margin of clypeus shallowly emarginate; malar space length 0.6× eye height; eyes separated by 1.3× their height; eye height 1.3× width. Antennae (Fig. 9) inserted slightly above lower margin of eyes, scape just reaching median ocellus, little shorter than eye; pedicel plus flagellum length 0.9× head width; pedicel as long as three anelli and Fl combined; clava much wider than funicle, length 2× width, as long as 3.5 preceding segments combined.

Mesosoma: (Figs 9 and 10) moderately reticulate, reticulation finer of scutellum and axillae. Pronotal collar not carinate Mesoscutum width 1.6× length. Scutellum very little convex, medially 0.8× as long as mesoscutum. Propodeum (Fig. 11) medially 0.5× as long as scutellum, shiny, median carina and plicae sharp but indistinct beyond middle; spiracles small and round. Metapleuron almost shiny. Forewing with basal cell and basal vein bare, discal pubescence moderately dense. Relative lengths: SMV 21.5, MV 10.5, PMV 7, STV 4.5.

Gaster: long, oval dorsally collapsing, little longer than head plus mesosoma combined; hypopygium reaching little beyond half length of gaster.

Holotype: Female, India, Kerala, Palghat district, Silent valley, 30.xii.1988, Coll. P. M. Sureshan.

Remarks: In colour, general morphology and nature of antenna this species resembles *M. teliformis* (Walker) but differs from it in having scape reaching median ocellus, temple length 0.6× eye length, malar space length 0.6× eye height and gaster only little longer than head plus mesosoma combined (1.1×) (in *teliformis* scape not reaching median ocellus, temple nearly or quite one third as long as the eye, malar space 0.4–0.5× eye height and gaster 1.25–1.5× as long as head plus mesosoma combined).

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Assessment of *Bacillus sphaericus* resistance in mosquitoes

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ABSTRACT: In the present study, the stability of *Bacillus sphaericus* (Bs) resistance is examined by subjecting selection pressure at three different levels (330, 660 and 990 mg toxin/lit) to *Bacillus sphaericus* resistance larvae of *Culex quinquefasciatus* Say, that have been reared in the laboratory for the last five years. Resistance ratio (RR) was calculated by comparing the susceptibility values between *B. sphaericus* resistance and susceptible larvae. We found that, the resistance was unstable by subjecting selection pressure with differential toxin concentrations. In the first toxin concentration level (330 mg/lit), the resistance ratio was 6013, 5087.1 and 4852.9 folds at LC_{50}, LC_{90} and LC_{95} levels respectively. Similarly, the second concentration level (600 mg/lit), the resistance ratio was increased in the levels of 8990, 10631.9 and 11131.4 folds in these lethal concentration levels. Similar observation was also observed in third concentration level (990 mg/lit). Variable (unstable) resistance to *B. sphaericus* toxin in *Cx. quinquefasciatus* may be due to differential concentration of selection pressure.

KEYWORDS: *Bacillus sphaericus*, *Culex quinquefasciatus* resistance, selection pressure, stability

INTRODUCTION

Bacillus sphaericus (Bs) is a potential bioinsecticide against several species of vector mosquitoes. Its main toxicity against *Culex* and *Anopheles* mosquito larvae is due to protein crystal inclusions produced during sporulation (Charles *et al.*, 1996). The crystal toxin is composed of two polypeptides with molecular masses of approximately 51 kDa and 42 kDa proteins (Arapinis *et al.*, 1988; Berry *et al.*, 1989). The crystals are ingested by the larvae and after solubilization and proteolytic cleavage, the activated toxin interacts with the midgut epithelium, leading to death of susceptible larvae (Broadwell and Baumann, 1987; Davidson *et al.*, 1987; Nicolas *et al.*, 1990). The

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toxin binding mechanism to a specific receptor sites in midgut brush border membrane (MBBM) of mosquitoes were also elucidated (Nielsen-LeRoux and Charles, 1992).

Though *B. sphaericus* toxins are powerful tool to control mosquitoes, the usage of this bioinsecticide is limited due to development of resistance by the susceptible mosquito species (Sinegre *et al.*, 1994; Rao *et al.*, 1995; Silva-Filha *et al.*, 1995; Poopathi *et al.*, 1999a). A low tolerance to *Bti* toxin in *Cx. quinquefasciatus* was also reported recently (Poopathi *et al.*, 1999b). So, examining the stability of resistance against *B. sphaericus* toxin in particular to filarial vector of *Cx. quinquefasciatus* may give basis for development of better methods to prevent or delay resistance problem in mosquito control operations. We undertook this study to evaluate the stability of *B. sphaericus* resistance in resistant strain of *Cx. quinquefasciatus* by subjecting selection pressure in different toxin concentration levels.

Culex quinquefasciatus third larvae (susceptible) were used from a colony maintained for more than five years, in the laboratory of CRME, Madurai and named as Madurai susceptible strain, (MS). The eggs and larvae collected from the field (Meenambikainagar, Madurai) were used to establish this colony.

A *B. sphaericus* resistant colony of *Cx. quinquefasciatus* collected from the field (Gandhinagar, Kochi, Kerala, S. India), where resistance has been reported (Rao *et al.*, 1995; Poopathi *et al.*, 1999a,b) was used in the present study. This resistant colony (named as Gandhinagar resistant strain, GR) has been submitted to selection pressure in three different concentrations (thousand early third instar larvae were treated at a concentration of 330, 660 and 990 mg.lit individually in 3 litre capacity bowl to derive the mortality of larvae by after 48 hr. The surviving larvae were reared to next generation to examine the stability of *Bs* resistance.

Both resistant (GR) and susceptible (MS) colonies were reared in the laboratory at ambiant laboratory temperature (29–314 °C) in enamel trays providing yeast and god biscuit at the ratio of 40 : 60 in water as nutrient source. Pupae were allowed to emerge in cages and the adults were sexed. Females were provided with blood mean from live chicken and males were provided with 2–5% glucose solution through cotton pads and water soaked raisin. Adults were allowed to oviposit in water in enamel cups kept inside the reading cages. Freshly hatched larvae from egg rafts of two larval strains (GR, MS) were cultured separatively as cyclic colonies.

Lyophilized bacterial culture of *B. sphaericus* 2362 (SPH-88) (titre : 1500 International toxic units/mg *Bs* toxin) received from Institute Pasteur, Paris, France was used as toxin source for selection pressure and bioassays. Titration and preparation of stock solution from *B. sphaericus* and bioassays were made as described in WHO protocol (Anonymous, 1985). In the present study, 5.50, 12.0 and 19.0 grams of *Bs* spore/crystal toxin were homogenized individually in appropriate volume of deionized water and stock solutions were prepared. The aliquots of appropriate dilutions ranging from 30 to 0.15 gm/lit and from 28 to 0.44 mg/lit were used for GR and MS strains respectively to monitor *B. sphaericus* resistance. Bioassays were conducted in disposable polythene cups (200 ml capacity). Test medium was prepared by adding appropriate volume of *Bs* toxin in 150 ml of water and twenty freshly moulted third

instar GR and MS strains were introduced individually in each test concentration. Larval food was given for *Bs* resistant and susceptible larvae as recommended by WHO. At each test concentration, three trials were made and each trial consisted of two replicates. The larval mortality was observed after 48 hours in *Bs* treated larvae. Control larval mortality was scored after 48 hrs and corrected for any control mortality by adopting Abott's (1925) formula:

$$\text{Corrected control mortality} = \frac{\%\text{ test mortality } \%\text{ control mortality}}{100\%\text{ control mortality}} \times 100$$

Moribund larvae if any, were counted as dead. The software package 'ASSAY' (courtesy of Dr. C. F. Curtis, London School of Tropical Medicine and Hygiene, U. K.) was used for dosage mortality regression analysis. Resistance ratio (RR) at LC_{50}, LC_{90} and LC_{95} levels were calculated by the method of Robertson and Preisler (1992).

$$\text{Resistance ratio} = \frac{LC_{50}/LC_{90}/LC_{95} \text{ of } Bs - \text{ resistant strain}}{LC_{50}/LC_{90}/LC_{95} \text{ of } Bs - \text{ susceptible strain}}$$

In the present study, stability of *B. sphaericus* resistance in *Cx. quinquefasciatus* resistance to *Bs* toxin was evaluated by subjecting selection pressure at three different concentration levels ie., 330, 660 and 990 mg/lit. A *B. sphaericus* susceptible strain of *Cx. quinquefasciatus* was used as control. Table 1 presents probit regression analysis on resistance ration (RR) between resistant GR) and susceptible (MS) strains. As shown in the table, in the first concentration (selection pressure) level (330 mg/lit) the LC_{50}, LC_{90} and LC_{90} and LC_{95} levels in *Bs* susceptible strain (MS) were 0.285, 1.47 and 2.34 mg *Bs*/lit respectively and it was found to be very low. Whereas, the LC_{50}, LC90 and LC_{95} levels in *Bs*-resistant strain (GR) were found to be very high in the levels of 1713.71, 7478.06 and 11355.9 mg *Bs*/lit respectively. The resistance ratio (RR) between GR and MS strains in these lethal concentration levels were 6013, 5087.1 and 4852.9 folds respectively. Thus, the results indicated clearly that, resistance was found to be very high in *Cx. quinquefasciatus* larvae, when subjected to selection pressure at 330 mg/lit as toxin concentration with *Bs* toxin. The variation in resistance ratio was seen among the lethal concentration levels (LC_{50}/ LC_{90}/ LC_{95} levels) its is expected that there may be variations in RR, since, the percentage mortality of larvae between the test concentrations were high in the GR strain than the MS strain (data not shown). However, statistically no significant difference in resistance ratio was observed in all lethal concentration levels, since the fiducial limits were overlapping. Table 1 also represents a similar probit regression analysis on resistance ratio between GR and MS strains by exposing the larval strain in the second concentration level (660 mg/lit). Here also, the LC_{50}, LC_{90} and LC_{95} levels, in *Bs*-susceptible strain were 0.426, 1.90 and 2.908 mg *Bs*/lit respectively. Whereas, in *Bs*-resistant strain, these lethal concentration levels were significantly increased to 3829.86, 20200.7 and 32380 mg/lit respectively. The corresponding resistance ratio between GR and MS strains in three lethal concentration levels were 8990, 10631.9 and 11131.4 folds respectively. Similar results were also observed in the resistant strain when they were treated at a concentration of 990 mg *Bs*/lit as shown in Table 1.

TABLE 1. Stability of *Bacillus sphaericus*[a] resistance in *Culex quinquefasciatus* by subjecting selection pressure at different concentration levels

Selection pressure (MG/Lit)	Mosquito strains	Intercept	Slope ± SE	LC_{50} 48hr (mg/lit) (95% FL)	(LC_{50} 48hr (mg/lit) (95% FL)	(LC_{95} 48hr (mg/lit) (95% FL)	χ^2 (df)	RR (at LC_{50})[e]	RR (at LC_{90})[e]	RR (at LC_{95})[e]
	MS[b]	5.98	1.79 ± 0.27	0.285	1.47	2.34	5.04(4)			
				(0.332 – 0.244)[d]	(0.92 – 1.12)[d]	(3.26 – 0.68)	5.04(4)			
330	GR[c]	1.48	2.003 ± 0.4	1713.71	7478.06	11355.9	21.6(4)	6013	5087.1	4852.9
				(2718.61 – 10.80.26)	(17722.6 – 3155.4)	(31873.5 – 40.45.89)		(11141.8 – 3253.8)	(15823.7 – 1634.9)	(18972.3 – 1241.1)
	MS	5.73	1.97 ± 0.27	0.426	1.90	23.908	5.95(4)			
				(0.49 – 0.37)	(2.46 – 1.47)	(3.96 – 2.14)				
660	GR	1.36	1.77 ± 0.23	3829.86	20200.7	32370.0	5.88(5)	8990.3	10631.9	11131.4
				(4447.0 – 3298.4)	(26803.6 – 15224.3)	(45296.1 – 23132.7)		(12018.9 – 6731.4)	(18233.7 – 618.7)	(21166.4 – 5841.6)
	MS	5.59	2.14 ± 0.29	0.532	2.107	3.11	1.71(4)			
				(0.607 – 0.466)	(2.67 – 1.66)	(4.14 – 2.34)				
990	GR	2.06	1.89 ± 0.27	5311.82	25221.86	39229.31	1.44(4)	9984.6	11970.5	12613.9
				(6130.7 – 4602.3)	(32832.4 – 19375.5)	(53885.9 – 28559.2)		(13156 – 7582)	(19778.5 – 7256.7)	(23028.1 – 6898.4)

[a]*Bacillus sphaericus* 2362 (SPH-88) = 1500 International toxic units/mg *Bs* toxin; Mosquito strains. [b]MS = Madurai susceptible strain; [c]GR - Gandhinagar resistant strain. [d]95% fiducial limits of upper and lower at different lethal concentration levels. [e]Resistance ratio $= \frac{LC_{50}/LC_{90}/LC_{95} \text{ from GR strain}}{LC_{50}/LC_{90}/LC_{95} \text{ from MS strain}}$.

Studies elsewhere have reported (Rodcharoen and Mulla, 1994; Rao *et al.*, 1995) a high level of resistance to *B. sphaericus* in California and Indian strain of *Cx. quinquefasciatus*. Poopathi *et al.* (1999a) have also reported cross-resistance to different strains of *B. sphaericus* in *Cx. quinquefasciatus* larvae. In the present study, a critical consideration of stability of *Bs* resistance was evaluated by subjecting selection pressure with different concentration of *Bs* toxin in *Cx. quinquefasciatus* larvae. It was observed that the resistant level was increased significantly with increasing concentration of toxin. We presumed that this variable (unstable) resistance to *B. sphaericus* toxin in *Cx. quinquefasciatus* might be due to inconsistant dose of toxin applied for selection pressure. The exact reason for conversion of *Bs* resistance in to susceptible one and resistance instability are need to be explored. Our results corroborate well with the findings of similar experiments with *Cx. quinquefasciatus* (Brazilian strain) (Silva-Filha and Regis, 1997). It is worthwhile to mention here that resistance to *B. sphaericus* in *Cx. pipiens pipiens* (French strain) is encoded by a single major recessive gene on linkage group I at 22.1 recombination units from the sex locus, (Nielsen-LeRoux *et al.*, 1997). Thus, we assume that the resistance instability by differential selection pressure may be due to impact of recessive gene from the sex locus of *Bs* resistant larvae. Further, there is a strong correlation between the mode of action of *B. sphaericus* toxin and resistant mechanism in mosquitoes. Where, the changes in the binding characteristics of toxin to receptor sites in resistant and susceptible larvae were studies extensively (Nielsen-LeRoux and Charles, 1992; Nielsen-LeRoux *et al.*, 1995, 1997; Silva-Filha *et al.*, 1997). This preliminary experiment may indirectly permit a possibility for developing unstable resistance in the field by the application of bioinsecticidal toxins at different concentration levels. Thus our results perhaps indicates a strong basis for seeking better methods to prevent or delay resistance problem in vector control operation, since the characteristics of resistance is considered to be unstable.

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Effect of botanicals on oviposition, hatchability and mortality of *Callosobruchus maculatus* L. (Coleoptera: Bruchidae)

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ABSTRACT: Six botanicals commonly found in home gardens in Sri Lanka were tested against the bruchid *Callosobruchus maculatus* (Coleoptera: Bruchidae) in the laboratory. *Ocimum sanctum* (Willd) significantly reduced egg deposition and hatchability followed by *Leucas zeylanica* L. Benth. The results of the study indicated the potential for using *O. sanctum* as a toxicant agent in storage against grain legume insect pests. It was also found that Cloves *Eugenia caryophyllata* L. powder gave highest mean number of adult mortality.

KEYWORDS: botanicals *Callosobruchus maculatus*

INTRODUCTION

Grain legumes constitute an important component of the diet of majority of the people of South East Asia, It is a source of dietary proteins. These grain legumes are attacked by many insects in the field and under storage conditions. The pulse beetle, *C. maculatus* is an important pest of grain legumes. Though infestation begins in the field serious damage is done to the pods and/or seeds during storage (Warui, 1984). This problem is serious at small-scale farmer level, village traders and average households where storage conditions are inadequate. Although insecticides have been widely used to protect grains from insect infestation, the concern over the development of resistant strains toxic residues, workers safety and prohibitive costs associated with insecticides have given impetus to the search for alternative methods of pest control.

Traditionally used by the farmers in the developing countries plant products appear to be quite safe and promising. Several authors have reported the insecticidal action and growth inhibiting effects of plant products on *C. maculatus* (Sowunmi and Akinnusi, 1983; Ajibola, 1975; Bhaduri *et al.*, 1985).

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TABLE 1. Mortality of *Callasobruchus maculatus* admixed with various doses of plant powder

Treatment	Dose (g)	Mortality
Control		2.087^{ef}
Flour		2.800^{d}
Papaya roots	0.250	3.350^{bc}
	0.500	3.750^{bc}
	1.000	4.800^{a}
	1.250	3.850^{b}
Clove leaves	0.250	4.917^{a}
	0.500	4.917^{a}
	1.000	4.917^{a}
	1.250	4.800^{a}
Cinnamon leaves	0.250	2.567^{de}
	0.500	2.700^{de}
	1.000	3.133^{cd}
	1.250	2.567^{de}
Ginger rhizome	0.250	2.767^{d}
	0.500	3.590^{bc}
	1.000	3.900^{b}
	1.250	1.650^{f}

Means with same letter are not significantly different according to DMRT.

In this study, various parts of indigenous plant species commonly available in home gardens in Sri Lanka were tested for their pesticidal properties against the pulse beetle *C. maculatus*.

Cowpea (variety E17A) was bought from the Department of Agriculture and was handpicked to remove infested seeds and other debris. These seeds were bulked, and transferred into polythene bags and incubated at ambient conditions (25–30 °C and 70–80% RH) in the laboratory for about 2 weeks before the bioassay.

Leaves of lemon grass (*Cymbopogan citratus* Stapf.), citronella (*Cymbopogan nardus* Linn.), getathumba (*Leucas zeylanica* (L.) Benth), maduruthala (*Ocimum sanctum* L.) cinnamon (*Cinnamomum verum*) and cloves (*Eugenia caryophyllata* L.), rhizomes of ginger (*Zingiber officinale* L.) and turmeric (*Curcuma longa* L.), cinnamon seed and papaya (*Carica papaya* L.) roots were used in the study. The materials were air dried under laboratory conditions (25–30 °C and 70–80% RH) and ground into fine powder on a laboratory mill and then passed through a mesh size of 0.25 mm wrapped in polythene bags until it is needed.

The laboratory culture of *C. maculatus* was used in both experiments from the culture maintained in the Department.

Six botanicals (Citronella, Lemon grass, Turmeric, Getathumba, Maduruthala and Cinnamon seed) were tested for their inhibitory effect on oviposition by *C. maculatus*.

TABLE 2. Mean oviposition of *Callasobruchus maculatus* after 3 DAT on cowpea seeds admixed with various dose of plant powders

Botanicals used	Dosage (g)	Mean Oviposition (1 DAT)	Mean Oviposition (3 DAT)
Control	0.00	45.133^{cde}	101.330^{fgh}
Flour	0.00	42.250^{cdef}	102.650^{fgh}
Citronella	0.25	47.000^{cde}	120.000^{cde}
	0.50	35.000^{efgh}	110.000^{defg}
	0.75	22.000^{ghi}	76.000^{i}
	1.00	54.000^{bc}	148.000^{b}
Lemon grass	0.25	42.000^{cdef}	113.000^{def}
	0.50	66.667^{b}	94.667^{h}
	0.75	66.000^{b}	147.500^{b}
	1.00	53.000^{bcd}	129.000^{c}
Turmeric	0.25	85.000^{a}	180.000^{a}
	0.50	54.000^{bc}	96.000^{gh}
	0.75	65.000^{b}	124.000^{cd}
	1.00	37.000^{defg}	108.000^{efgh}
Gatathumba	0.25	40.000^{cdef}	79.000^{hi}
	0.50	26.000^{fghi}	50.000^{ij}
	0.75	21.000^{ghi}	57.000^{ij}
	1.00	35.000^{efgh}	76.000^{i}
Maduruthala	0.25	22.000^{ghi}	76.000^{i}
	0.50	19.000^{hi}	55.000^{ij}
	0.75	15.000^{i}	35.000^{k}
	1.00	25.667^{fghi}	42.000^{jk}
Cinnamon seed	0.25	45.000^{cde}	109.000^{efgh}
	0.50	26.000^{fghi}	72.000^{i}
	0.75	31.667^{efgh}	104.000^{fgh}
	1.00	42.000^{cdef}	56.000^{ij}

Means with same letter are not significantly different at 5% level.

Each powder was tested at four doses (0.25, 0.5, 0.75 and 1 g). Each powder was added to 100 seeds of cowpea in 150 ml translucent plastic cups and the powders were spread over the seeds by vigorously shaking the cups. Two control treatments were maintained; one without any powder and the other with flour. Newly emerged *C. maculatus* adults 0–24 hrs (5 males and 5 females) were added into each cup, covered firmly with cheese cloth and kept in the laboratory at ambient conditions. Each treatment was replicated 10 times. The number of eggs laid at 1, 3, 5 and 10 days after treatment (DAT) were counted.

Four botanicals (Papaya roots, cinnamon leaves, clove leaves and ginger rhizomes) were tested for adult mortality. Testing of the botanicals was done as described in experiment 1. Each treatment was replicated 10 times. The mortality of adults was

TABLE 3. Mean number of hatched eggs accumulated after 5 and 10 DAT on cowpea seeds admixed with various doses of plant powders

Botanicals used	Dosage (g)	(5 DAT)	(10 DAT)
Control		44.050^{cdef}	83.05^{h}
Flour		38.300^{defgh}	102.00^{f}
Citronella	0.25	29.000^{efghijk}	104.00^{f}
	0.50	21.000^{ghijk}	125.00^{de}
	0.75	54.000^{bcd}	90.00^{gh}
	1.00	46.000^{cde}	140.00^{bc}
Lemon grass	0.25	56.000^{bcd}	120.00^{e}
	0.50	90.333^{a}	131.00^{cd}
	0.75	64.500^{b}	155.50^{a}
	1.00	61.667^{bc}	154.00^{a}
Turmeric	0.25	40.000^{defg}	149.00^{ab}
	0.50	26.000^{fghijk}	80.00^{hi}
	0.75	29.000^{efghijk}	90.00^{gh}
	1.00	22.667^{ghijk}	89.00^{gh}
Gatathumba	0.25	34.000^{efghi}	62.00^{jk}
	0.50	28.000^{efghijk}	69.00^{jk}
	0.75	14.000^{jk}	89.00^{gh}
	1.00	31.667^{efghij}	60.00^{k}
Maduruthala	0.25	26.000^{fghijk}	84.00^{h}
	0.50	14.667^{ijk}	70.00^{ijk}
	0.75	12.000^{k}	40.00^{l}
	1.00	16.000^{ijk}	48.00^{l}
Cinnamon seed	0.25	31.000^{efghijk}	91.00^{gh}
	0.50	20.000^{hijk}	72.00^{ij}
	0.75	29.000^{efghijk}	98.00^{fg}
	1.00	30.000^{efghijk}	134.00^{cd}

Means with same letter are not significantly different at 5% level.

observed 4 DAT. Adult insects were sorted out of the powdered material and were counted. The mortality data was corrected using Abbots formula before ANOVA.

Among the plant powders tested, Maduruthala (*O. sanctum*) was the most effective treatment for suppressing oviposition significantly (Table 2) followed by Getathumba (*L. zeylanica*). Ocimum species are known to be important sources of a repellent and toxicant against major insect pests. The repellency of eugenol extracted from the leaves of *O. suave* (Willd) against *Sitophilus zeamais* (Mots.) in laboratory bioassays has already been reported (Hassanali *et al.*, 1990). Weaver *et al,* (1991) reported the efficacy of milled dried leaves of *O. canum* (Sims) for the protection of edible beans against damage by *Zabrotes subfasciatus* (Bohem).

The other plant powders tested did not significantly affect hatchability (Table 3). The ineffectiveness of the powders of some of the plants studied could be partly due to the fact that the insecticidal compounds in the plant might be volatile in nature and might have been lost during drying and powdering of the leaves. There was also evidence of oviposition attractancy with some of the powders; as evident in (Table 2) with turmeric at 0.25 concentration.

Clove powder was the most effective treatment, among the 4 powders tested for adult mortality followed by root dust papaya (Table 1). The other two botanical powders tested in this experiment did not give significant results when compared with control. Ivbijaro and Agbaje (1986) recorded high bruchid mortality in cowpea seeds treated with *Piper quineense* powder at 1.0 and 1.5 g per 20 g. Su (1977) showed that cause of high mortality of adults exposed to high does of *P. quineense* could be due to either stomach toxicity or contact toxicity. The adult mortality caused by clove powder may be due to its chemical components as reported by Su (1977) on *P. quineese*. We believe that the identification of active compounds and their mode of action would contribute to their resourceful use in village storage crop protection.

Botanical pesticides will become an important factor in integrated pest management programmes in developing countries like Sri Lanka since these natural pesticides are selective and safe. Appropriate technology transfer system should be developed to promote direct preparation of these pesticides at farmers fields, if it benefits poor farmers.

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Improvement in economic characters of silkworm, *Bombyx mori* L. by Folic acid administration

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ABSTRACT: The frolic acid was administered orally with mulberry leaves to the fifth instar larvae of *Bombyx mori* (PM × NB_4D_2) at 0.005, 0.05 and 0.5 mg/ml concentrations, once a day till the onset of the spinning of cocoons. It was found nutritionally important as the larvae gained weight with substantial reduction in larval duration ($P < 0.01$) with all the three concentrations. Administration of 0.5 mg/ml folic acid concentration resulted significant increase in all the major economic characters such as weight of cocoon (12.2%), shell (21.9%), silk filament length (17.8%) and weight (23.0%) and denier (4.5%). Folic acid probably facilitated nucleic acid synthesis in the silk gland cells and thereby improved the absolute silk content in the shell. The significant increase in fecundity by 32.6% with folic acid also suggests its role in the process of oogenesis and yolk formation in *B. mori*.

KEYWORDS: Silkworm, *Bombyx mori*, Folic acid, economic characters, fecundity

It is now well established that all the insects require from their diet most of the B vitamins particularly thiamin (B_1), riboflavin, nicotinic acid, pyridoxine (B_6), pantothenic acid, folic acid (or folinic acid) and biotin. Among these, folic acid is a compound made up of the pteridine nucleus, P-aminobenzoic acid and glutamic acid (Harper, 1977). Tetrahydrofolate derived from folic acid is necessary for nucleic acid biosynthesis and dietary nucleic acid can spare the folic acid requirement of the flies, *Drosophilla melanogaster* (Sang, 1978) and *Musca domestica* (Perry and Miller, 1965). Further, the folic acid has other functions than nucleic acid synthesis. It is proved by showing the inability of nucleic acid to support normal growth of housefly larva fed on a diet containing the aminopterin, a inhibitor of dihydrofolic acid (Perry and Miller, 1965).

In *Bombyx mori*, although Horie *et al.* (1966) have reported the requirement of some of the B vitamins for its normal growth, information on the requirement and role of folic acid in silkworm in relation to larval growth and economic characters is scanty.

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This paper describes the effect of oral folic acid administration on the larval growth and economic characters of *B. mori*.

The silkworm larvae (PM × NB_4D_2) were reared on Kanva-2/M5 mulberry leaves during the month of November and February following the method of Krishnaswami (1986). About 1200 freshly moulted fifth instar larvae were selected from the stock culture and distributed in 12 wooden rearing trays (3 × 2 ft.), each containing 100 larvae. The trays were further divided into four groups, each of 3 trays. Three solutions of 0.005, 0.05 and 0.5 mg/ml concentrations of commercially available Folic acid (Swiss Pharama Pvt. Ltd., Ahamadabad, India) were prepared in distilled water.

Fresh matured mulberry leaves of uniform size were plucked and smeared with 10ml solution of each concentration of folic acid, separately. The leaves smeared with each concentration of folic acid were air-dried and provided to the larvae in 3 trays of respective group, once a day. The leaves smeared with 10ml of distilled water were provided to the control larvae, once a day. For remaining three scheduled feedings, known quantity of fresh leaves was provided. This feeding schedule was continued till the onset of spinning and the experiment was repeated thrice. Larval growth was determined by weighing the larvae on 1st, 3rd, 5th and 7th day during fifth instar stage. The total duration of survived fifth instar larvae of each group was recorded.

Economic characters were assessed using cocoons harvested on the fifth day of spinning. Cocoon and shell weights were recorded by weighing 25 cocoons from each group individually and shell ratio was calculated. About 20 cocoons from each group were cooked and reeled individually using Approuvet. The length and weight of absolute silk filament were obtained and denier was calculated. The moths emerged from the remaining cocoons of treated and control groups were allowed to lay eggs after mating. The eggs produced by each female of both treated and control groups were recorded. The data obtained were analyzed statistically using t-test.

The folic acid administration to the newly moulted fifth instar silkworm larvae of PM × NB_4D_2 hybrid in different concentrations (0.005, 0.05 and 0.5 mg/ml) once a day enhanced the larval growth from third day onwards. It was evidenced by increase in larval weight ranging from 3.61–3.8 times in folic acid administered groups, whereas in control the increase was 3.39 times on 7th day (Table 1). Consequently, the larval duration of 5th instar was reduced significantly ($P < 0.001$) from 8–18 h in folic acid administered groups when compared with control (Table 1). The survival rate remained more than 99% in both control and treated groups (Table 1).

The cocoon and shell weights were significantly increased in all the groups administered with folic acid concentrations. The highest increase in cocoon and shell weight was 12.2 and 21.9%, respectively with 0.5 mg/ml concentration. The shell ratio was also high in the treated groups than control (Table 2). A significant increase in the silk filament length and its weight was evident in all the treatments. The maximum increase in silk filament length and its weight was about 17.8% and 23.0%, respectively with 0.5 mg/ml folic acid concentration. The denier of the silk filament was higher with all the three concentrations of folic acid than control, but it was

TABLE 1. Effect of folic acid administration on larval growth, duration and survival of *B. mori*.

Treatment	Concentration (mg/ml)	Weight of 10 larvae (g) on Days				Larval Duration (h)#	Survival (%)
		1	3	5	7		
Control	Distilled water	8.04	16.33 (2.03)	23.97 (2.98)	27.22 (3.39)	214 ± 0.9	99
Folic Acid	0.005	7.61	14.44 (1.90)	24.54 (3.22)	28.60 (3.76)	206 ± 1.0*	100
"	0.05	8.45	17.23 (2.04)	27.45 (3.25)	30.45 (3.61)	203 ± 0.9*	100
"	0.5	7.35	15.12 (2.06)	25.46 (3.46)	27.95 (3.80)	1.96 ± 0.9*	99

- Values are Mean ± SE; figures in parentheses denote weight increase in times;
Statistically significant at * $P < 0.001$.

TABLE 2. Effect of folic acid administration on economic characters and fecundity of *B. mori*.

Treatment	Concentration (mg/ml)	Cocoon Weight (g)#	Shell		Silk filament		Denier#	Fecundity#
			Weight (g)#	Ratio (%)	Length (m)#	Weight (g)#		
Control	Distilled water	1.409 ± 0.021	0.228 ± 0.007	16.2	608.4 ± 13.7	0.165 ± 0.005	2.44 ± 0.03	463 ± 09
Folic Acid	0.005	1.465 ± 0.023*	0.245 ± 0.005*	16.7	649.9 ± 12.9*	0.180 ± 0.002***	2.50 ± 0.04	506 ± 12**
"	0.05	1.492 ± 0.017*	0.254 ± 0.005**	17.0	658.8 ± 14.2**	0.185 ± 0.003***	2.53 ± 0.03*	552 ± 17***
"	0.5	1.581 ± 0.019**	0.278 ± 0.005**	17.6	716.5 ± 11.3***	0.203 ± 0.002***	2.55 ± 0.03*	614 ± 15***

- Values are Mean ± SE; Statistically significant at *P < 0.05,**P < 0.01, ***P < 0.001.

significantly high ($P < 0.05$) with 0.05 and 0.5 mg/ml (Table 2). The highest increase in egg production was 32.6% with 0.5 mg/ml folic acid concentration.

At least three chemically related compounds of folic acid are nutritionally important which occur in natural products. These compounds differ only in the number of glutamic acid residues attached to the pteridine-aminobenzoic acid complex (Harper, 1977). In the present study, the administration of commercially available folic acid to fifth instar larvae of *Bombyx mori* exhibited its nutritional value by increasing larval weight and significant reduction in larval duration. Although Horie *et al.* (1966) were unable to demonstrate the amount of folic acid requirement in *B. mori*, the functions of folic acid for the support of normal growth in housefly have been reported by Perry and Miller (1965). They suggested that either there are sufficient egg reserves of folic acid to fully support other folate functions or intrinsic folate synthesis in the housefly, *M. domestica*. The latter possibility is supported by direct evidence of folate synthesis in *D. melanogaster* and *Aedes aegypti* (Venters, 1971). This possibility can not be ruled out in *B. mori*, since folates are present in a wide variety of plants mainly as polyglutamates in reduced methyl or formyl forms (Harper, 1977).

Harper (1977) reviewed that the folic acid coenzymes are specifically concerned with several important biochemical reactions and nucleic acid synthesis. In the present study, the significant enhancement in cocoon and shell weight, silk filament length and its weight and denier, indicates that folic acid also plays an important role in improvement of economic characters of *B. mori*. It has been estimated that during ontogenesis of *B. mori*, the cells of anterior, middle and posterior regions of silk gland undergo 13, 19–20 and 18–19 rounds of DNA duplication, respectively (Perdrix-Gillot, 1979). Since the folic acid is necessary for nucleic acid biosynthesis in *M. domestica* and *D. melanogaster* (Perry and Miller, 1965; Sang, 1978), the extrinsic administration of folic acid in *B. mori* might have facilitated the nucleic acid synthesis in silk gland cells during growth and secretary phase of the silk gland, resulting into increase in silk synthesis and in turn high absolute silk content in the shell. Increase in the weight of larva, cocoon and shell and oviposition by diet supplementation with FE-PLUS (Ferrous fumerate + Folic acid) in *B. mori* has also been reported (Khan and Saha, 1996). A significant increase in egg laying by female due to folic acid administration also suggests its role in the process of oogenesis and yolk formation.

The present study thus reveals that oral administration of folic acid could improve cocoon weight up to about 12%, shell weight up to about 21%, absolute silk filament length 17% and its weight up to about 23%. In addition to this, egg production could be enhanced about 32% with folic acid administration in grainages.

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Effect of insecticides on sodium and potassium ions in the larvae of the coconut pest, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)

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ABSTRACT: Effect of Rogor and BHC on sodium and potassium ion levels in the digestive system and body wall of the third instar larvae of *Oryctes rhinoceros* was studies through Flame photometer by exposing them to different concentrations of above pesticides. The cations (Na^+ and K^+) have important role in membrane polarization and permeability by their differential distribution on either side of the cells. The concentration of Na^+ was found to increase at the initial stages of treatment in both Rogor body wall and digestive system which came down thereafter. On the other hand, the concentration of K^+ had decreased in the initial treatment which reached to minimum quantity later.

KEYWORDS: insecticides, ionic levels, *O. rhinoceros* larvae, body wall, digestive system, flame photometer

The rhinoceros beetle is one of the most serious pest of the coconut palm. Hence various investigations have been carried out to control them through insecticides applied to potential breeding placed including compost, decaying woods etc. Many attempts have been made to control *O. rhinoceros* using different types of pesticides, (Nirula *et al.*, 1955; Abraham and Kurian, 1970; Bedford, 1981; Visalakshi *et al.*, 1988; Babjan *et al.*, 1993; Jacob, 1996). Eventhough, control methods have been worked out on *O. rhinoceros* in detail no reports other than those of Sudarshan Kumar (1995), Ramanna (1992) and Rosetta (1997), Venkatarajappa and Krishnan (1999) are available on physiological changes taking place in the organisms at tissue level after treating them with insecticides. Hence, the present paper deals with changes in the levels of ions in the digestive system and body wall. Rogor and BHC insecticides are commonly used to control this pest at larval/adult stages.

Larvae of *O. rhinoceros* were collected from the field and acclimatized in the laboratory conditions using compost. The third instar larvae were selected for the present investigations, because of its abundance in the upper strata of the compost, this makes the easy accessibility of pesticides to the larvae. They were treated

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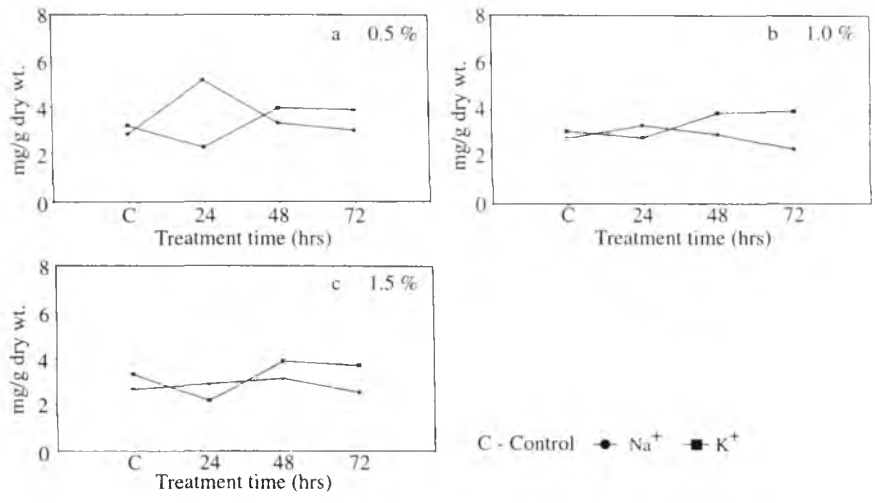

FIGURE 1. Effect of BHC on Na^+ and K^+ concentration in the digestive system of *O. rhinoceros* (III instar) larvae.

with two commonly used insecticides namely Rogor (organophosphorus) and BHC (organochlorine). These insecticides were mixed with compost to get the final concentrations (0.125, 0.25, and 0.5%) of Rogor at intervals of 8, 16 and 24 hours and 0.5, 1.0 and 1.5% of BHC at intervals of 24, 48 and 72 hours for each concentration.

To study the effect of pesticides on different tissues, digestive system and body wall were chosen, since the effect on those organs will be more conspicuous. To follow the changes in ionic level total Na^+ and K^+ concentration were analysed through flame photometer following the method of Jackson (1967).

A perusal of Fig. 1 and Table 1 indicates the changes taking place in Na^+ and K^+ concentrations of digestive system in response to treatment with BHC. When larvae were exposed to 0.5% BHC, an increase in the concentration of Na^+ was noticed after 24 hours of treatment (2.83–5.19 mg/g). After 24 hours it started declining reaching a value of 3.32 and 2.99 mg/g after 48 and 72 hours respectively. On the other hand, the concentration of K^+ had decreased to 2.26 from 3.21 mg/g (control) after 24 hours. Later, it showed an increase reaching a value of 3.96 mg/g. But, after 72 hours it reached a minimum quantity of 3.86 mg/g. As such the changes in Na^+ and K^+ concentrations appear to have an inverse relationship at the concentration of 0.5% BHC (Fig. 1(a)). A similar pattern was observed when the larvae were treated with 1.0 and 1.5% BHC (Fig. 1(b) and (c), Table 1). The changes in Na^+ and K^+ ions in body wall seems to be more or less similar to those found for the digestive sytem i.e. inverse relationship between Na^+ and K^+ ions in all concentrations of BHC (Fig. 2(a)–(c); Table 1).

When the larvae treated with 0.25 & 0.5% of Rogor the pattern of changes in Na^+ & K^+ concentration in digestive system showed an inverse relationship (Fig. 3(b)

TABLE 1. Changes in Na^+ and K^+ concentration (mg/g dry wt.) in the digestive system & bodywall of III instar larvae of *O. rhinoceros* treated with BHC

Tissue	Na^+ (control)	K^+ (control)	Concentration of BHC (%)	Time of treatment (hrs)	Changes in Na^+	Changes in K^+
				24	5.19	2.26
	2.83	3.21	0.5	48	3.32	3.96
				72	2.99	3.87
				24	3.36	2.82
Digestive system	2.79	3.10	1.0	48	2.98	3.89
				72	2.34	3.97
				24	2.94	2.19
	2.69	3.34	1.5	48	3.16	3.91
				72	2.57	3.71
				24	5.20	2.41
	3.03	3.94	0.5	48	3.67	4.11
				72	3.38	4.02
				24	3.61	3.77
Body wall	2.95	3.84	1.0	48	2.52	4.17
				72	2.35	4.28
				24	3.24	2.45
	3.05	3.92	1.5	48	3.38	4.11
				72	2.75	4.32

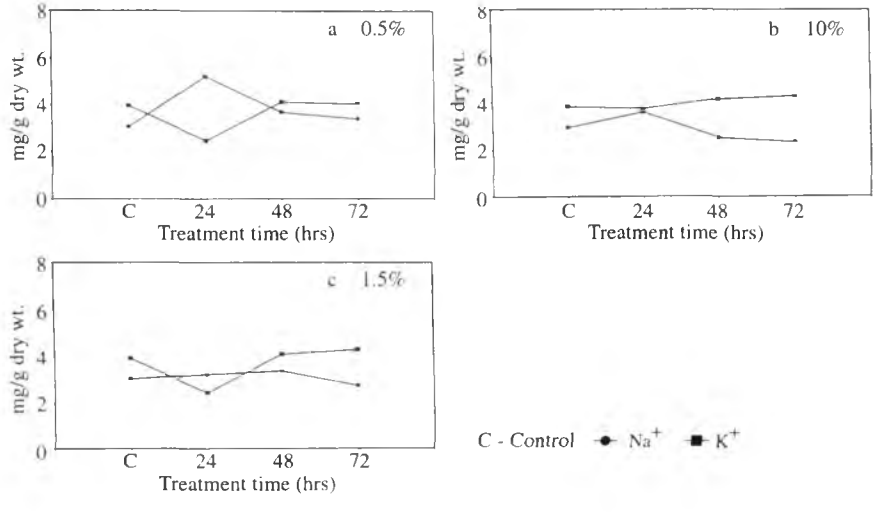

FIGURE 2. Effect of BHC on Na^+ and K^+ concentration in the bodywall of *O. rhinoceros* (III instar) larvae.

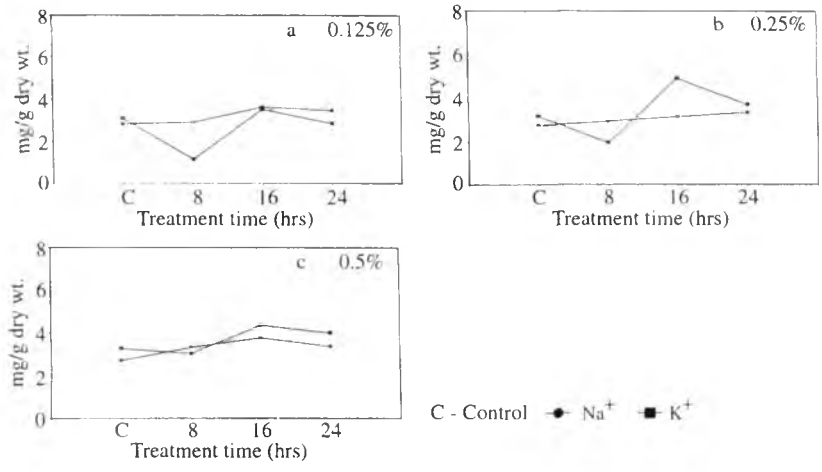

FIGURE 3. Effect of Rogor on Na^+ and K^+ concentration in the digestive system of *O. rhinoceros* (III instar) larvae.

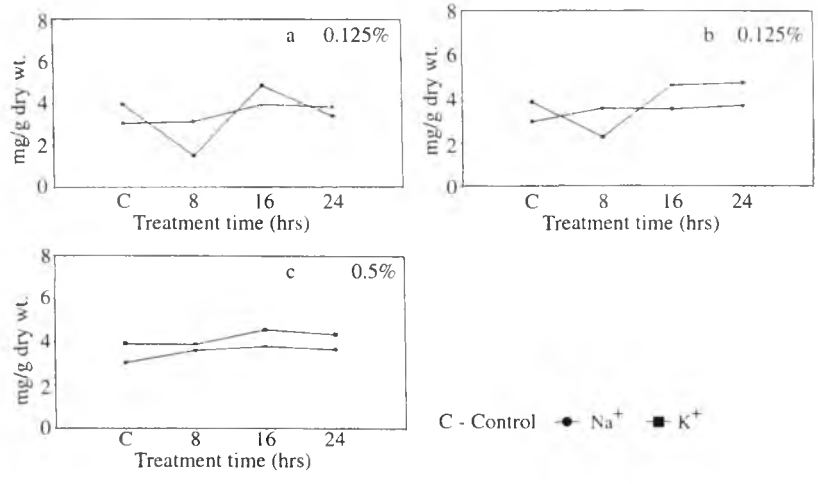

FIGURE 4. Effect of Rogor on Na^+ and K^+ concentration in the bodywall of *O. rhinoceros* (III instar) larvae.

and (c)). In case of 0.125% treatment no such relationship was prevalent (Fig. 3(b) and (c)). In case of 0.125% treatment no such relationship was prevalent (Fig. 3(a) and Table 2).

In case of body wall, no inverse relationship between Na^+ and K^+ ion concentration was evident in 0.125 and 0.5% Rogor (Figs 4(a) and (c)). However, when the larvae were treated with Rogor 0.25% there was an inverse relationship (Fig. 4(b) and Table 2).

TABLE 2. Changes in Na^+ and K^+ concentration (mg/g dry wt.) in the digestive system & bodywall of III instar larvae of *O. rhinoceros* treated with Rogor

Tissue	Na^+ (control)	K^+ (control)	Concentration of Rogor (%)	Time of treatment (hrs)	Changes in Na^+	Changes in K^+
				08	2.91	1.17
	2.83	3.10	0.125	16	3.61	3.51
				72	2.99	3.87
				08	2.99	1.99
Digestive system	2.79	3.22	0.25	16	3.21	4.98
				24	3.42	3.79
				08	3.31	3.01
	2.69	3.25	0.5	16	3.76	4.35
				24	3.37	3.99
				08	3.10	1.48
	3.03	3.94	0.125	16	3.95	4.85
				24	3.381	3.37
				08	3.54	2.25
Body wall	2.95	3.82	0.25	16	3.52	4.62
				24	3.67	4.70
				24	3.62	3.89
	3.05	3.92	0.5	48	3.81	4.57
				72	3.67	4.34

In the present investigation, changes in Na^+ and K^+ concentration have been analysed in digestive system and body wall in response to treatment with two commonly used insecticides namely BHC (organochlorine) and Rogor (organophosphorus). These two ions were specifically chosen, since they may indicate their activity at cellular level. Analysis of sodium and potassium were carried out, since they are the most important cations of physiological fluid and tissue, and also they play an important role in membrane polarization and permeability. Na^+ is the major constituent of extracellular compartment, while K^+ is preferentially found in higher concentration within the cellular compartment (Patel and Ramachandran, 1986). These were analysed in digestive system and body wall after treatment with BHC and Rogor, since the above tissues constitute the bulk of the organism where one can expect accumulation of the insecticides in them after exposure. An overall picture of cations show (Na^+ and K^+) a clear inverse relationship in different concentrations after treatment with BHC in body wall and digestive system. This was evident for digestive system and body wall after treatment with Rogor also. From the observations it is assumed that the insecticides alter the concentration of these cations, due to on the altered flow of them inside to the outside of cell and vice versa. In general, as a result of treatment with BHC and Rogor, the potassium concentration showed a declining pattern. This phenomenon could be due to a reduction in the volume of

cell (Padmasheela and Krishnan, 1998) and resulting in the reduction of potassium concentration (inside the cell). Eventhough, a continuous increase is not evident for sodium after treatment with either Rogor or BHC (for body wall and digestive system), an overall analysis showed an increase of sodium concentration at the initial stages of treatment. This is also not unexpected, since it would have resulted from an increase in the same in the extra cellular fluid. At the same time, an increase in the Na^+ concentration may indicate a failure of the transport mechanism, particularly the 'sodium pump'. The sodium pump is known to be linked to the metobolism of the cells. Hence, treatment with metobolic inhibitors like cyanide, dinitrophenol etc. stops the outward transport of sodium ions. A consequence of failure of the sodium pump is that continued passive diffusion inwards causes the internal concentration of to Na^+ increase (Edward and Hassel, 1980). In the same way, the insecticides might have been causative factors for the increase of Na^+ in the digestive system and body wall. The initial decrease in the K^+ could be due to a reduction in the cellular metabolic activity. However, the later increase of the same might be due to the 'physiological imbalance' or due to prolonged insecticide treatment (which could have acted as a metabolic inhibitor).

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BOOK NOTICE

Parasitic Hymenoptera and Biological Control

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The book contains keys to superfamilies with figures to most couplets. For each family a habitus drawing, a diagnosis, biological notes, major points on biological control, systematics ad pertinent references are provided. Classification of major divisions of Hymenoptera, classification of parasitic Hymenoptera, general morphology of parasitic Hymenoptera, details on collecting and preserving, packing and shipping etc are also provided. Besides these a separate chapter is provided on the importance of parasitic Hymenoptera in biological control of insect pests. The book will be a valuable source of reference concerning parasitic Hymenoptera, their host-parasite relationships and relation to biological control, not only to entomologists, agricultural scientists and biological control workers but also to post-graduate students of entomology. More than 120 illustrations are provided. The famous entomologist Professor M. S. Mani has written a 'Forward' to the book.

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